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UNITED STATES DISTRICT COURT
DISTRICT OF NEW JERSEY

BRISTOL-MYERS SQUIBB COMPANY, Plaintiff, v. DR. REDDY'S LABORATORIES, LTD. AND DR. REDDY'S LABORATORIES, INC., Defendants.	Civil Action No. _____ **COMPLAINT FOR PATENT INFRINGEMENT**

PLAINTIFF BRISTOL-MYERS SQUIBB COMPANY'S COMPLAINT

Plaintiff Bristol-Myers Squibb Company ("Bristol-Myers Squibb" or "Plaintiff"), having a principal place of business at 345 Park Avenue, New York, New York, 10154, brings this action for patent infringement against Defendants Dr. Reddy's Laboratories, Ltd. ("DRL Ltd."), having a principal place of business at Bachupally, 500 090, India and Dr. Reddy's Laboratories, Inc. ("DRL Inc."), having a principal place of business at 200 Somerset Corporate Blvd.,

Bridgewater, New Jersey 08807 (collectively, "DRL" or "Defendants"). Plaintiff alleges as follows:

NATURE OF THE ACTION

1. This is a civil action for patent infringement under the patent laws of the United States, Title 35, United States Code, arising out of Defendants' filing of Abbreviated New Drug Application ("ANDA") No. 20-4303 with the United States Food and Drug Administration ("FDA") seeking approval to manufacture and sell a generic version of Plaintiff's successful Ixempra® kit product prior to the expiration of U.S. Patent Nos. 6,670,384, 7,022,330 and RE41,393.

THE PARTIES

2. Plaintiff Bristol-Myers Squibb is a company organized and existing under the laws of the State of Delaware. Bristol-Myers Squibb operates multiple Research and Development sites, including sites in Lawrenceville, Hopewell, and New Brunswick, New Jersey, among others. Bristol-Myers Squibb manufactures and brings to market innovative medicines and technologies.

3. Upon information and belief, Defendant Dr. Reddy's Laboratories, Ltd. is an entity organized and existing under the laws of India. Upon information and belief, DRL Ltd. develops and markets generic drug products for sale and use throughout the United States, including for sale and use in the State of New Jersey.

4. Upon information and belief, Defendant Dr. Reddy's Laboratories, Inc. is a company organized and existing under the laws of the State of New Jersey. Upon information and belief, DRL Inc. markets a wide range of generic drug products and regularly conducts business throughout the United States, including in the State of New Jersey. Upon information and belief, DRL Inc. is a wholly-owned subsidiary of DRL Ltd.

5. Upon information and belief, DRL Ltd. prepared and submitted ANDA No. 20-4303 in collaboration with DRL Inc.

JURISDICTION AND VENUE

6. This action arises under the patent laws of the United States of America. This court has subject matter jurisdiction under 28 U.S.C. §§ 1331, 1338(a), 2201, and 2202.

7. This Court has personal jurisdiction over Defendants by virtue of the fact that, among other things, each Defendant has continuous and systematic contacts with New Jersey. Defendants have committed, or aided, abetted, contributed to and/or participated in the commission of acts of patent infringement that will lead to foreseeable harm and injury to Plaintiff, which manufactures numerous drugs for sale and use throughout the United States, including this judicial district. This Court has personal jurisdiction over each of the Defendants for the additional reasons set forth below and for other reasons that will be presented to the Court if such jurisdiction is challenged.

8. On information and belief, both DRL Ltd. and DRL Inc. have previously consented to personal jurisdiction in this judicial district in several cases as plaintiffs and defendants.

9. This Court also has jurisdiction over DRL Inc. because, among other things, it has a principal place of business in the State of New Jersey and thus has submitted itself to the personal jurisdiction of the courts in New Jersey.

10. Venue is proper in this judicial district under 28 U.S.C. §§ 1391(b), (c) and 1400(b).

BACKGROUND

11. Bristol-Myers Squibb developed and manufactures the Ixempra® kit pursuant to New Drug Application No. 022-065, which was approved by the FDA. The Ixempra® kit

delivers a microtubule inhibitor, and is indicated for both monotherapy and combination therapy breast cancer treatment. As a monotherapy, the Ixempra® kit is indicated for the treatment of metastatic or locally advanced breast cancer in patients after the failure of an anthracycline, a taxane, and capecitabine. As a combination therapy, the Ixempra® kit is indicated in combination with capecitabine for the treatment of metastatic or locally advanced breast cancer in patients after the failure of anthracycline and a taxane.

12. Upon information and belief, DRL seeks approval to market a generic version of the Ixempra® kit for at least one of the uses approved by the FDA.

13. United States Patent No. 6,670,384 (the "'384 patent"), entitled "Methods of Administering Epothilone Analogs for the Treatment of Cancer," was duly and legally issued by the United States Patent and Trademark Office on December 30, 2003, to inventors Bandyopadhyay et al. The '384 patent is owned by Bristol-Myers Squibb.

14. The FDA-approved product and use of the Ixempra® kit is covered by one or more claims of the '384 patent, and the '384 patent was listed in connection with the Ixempra® kit in the FDA's Approved Drug Products with Therapeutic Equivalence Evaluations, commonly known as the "Orange Book."

15. United States Patent No. 7,022,330 (the "'330 patent") entitled "Parenteral Formulation for Epothilone Analogs," was duly and legally issued by the United States Patent and Trademark Office on April 4, 2006 to inventors Bandyopadhyay et al. The '330 patent is owned by Bristol-Myers Squibb.

16. The FDA-approved product and use of the Ixempra® kit is covered by one or more claims of the '330 patent, and the '330 patent was listed in connection with the Ixempra® kit in the Orange Book.

17. United States Patent No. RE41,393 (the "'393 patent") entitled "Treatment of Refractory Tumors Using Epothilone Derivatives," was duly and legally reissued by the United States Patent and Trademark Office on June 22, 2010 to inventor Lee. The '393 patent is a reissue of United States Patent No 6,686,380, which was duly and legally issued by the United States Patent and Trademark Office on February 3, 2004 to inventor Lee. The '393 patent is owned by Bristol-Myers Squibb.

18. The FDA-approved use of the Ixempra® kit is covered by one or more claims of the '393 patent, and the '393 patent was listed in connection with the Ixempra® kit in the Orange Book.

19. Upon information and belief, DRL submitted ANDA No. 20-4303 under 21 U.S.C. § 355(j)(2) in order to obtain FDA approval to engage in the commercial manufacture, use, and/or sale of a generic version of the Ixempra® kit, to be used in infringing manners, prior to the expiration of the '384, '330 and '393 patents.

20. By letter dated November 13, 2012, Defendants notified Plaintiff that DRL had submitted ANDA 20-4303 concerning its proposed drug product of ixabepilone for injection ("DRL's ANDA product") as required by § 505(j)(2)(B)(ii) of the Federal Food, Drug and Cosmetic Act ("FDC Act"). *See* 21 U.S.C. § 355(j)(2)(B)(ii).

21. DRL's letter further notified Plaintiff that DRL had filed with the FDA, pursuant to § 505(j)(2)(A)(vii)(IV), a certification with respect to the '384, '330, and '393 patents ("Paragraph IV certification"), alleging, along with the letter, that the '384, '330, and '393 patents are invalid and/or will not be infringed by the commercial manufacture, use, offer for sale, or sale of DRL's ANDA product.

22. This action is being brought pursuant to 21 U.S.C. § 355(j)(5)(B)(iii) within forty-five days of Bristol-Myers Squibb's receipt of DRL's Notice Letter.

COUNT I
INFRINGEMENT OF U.S. PATENT NO. 6,670,384

23. Plaintiff re-alleges and incorporates by reference paragraphs 1–22, above.

24. Bristol-Myers Squibb is the owner by assignment of the '384 patent and has the right to sue for infringement thereof. A true and correct copy of the '384 patent is attached as Exhibit A.

25. DRL's ANDA product, or use thereof, would directly infringe one or more of the claims of the '384 patent.

26. DRL's submission of ANDA No. 20-4303 seeking approval for the commercial manufacture, use, offer for sale, and/or sale of DRL's ANDA product before the expiration of the '384 patent constitutes an act of infringement of one or more claims of the '384 patent under 35 U.S.C. § 271(e)(2)(A).

27. The commercial manufacture, use, offer for sale, sale and/or importation of DRL's ANDA product would infringe and/or contribute to and/or induce infringement of one or more claims of the '384 patent.

28. Upon information and belief, DRL intends to engage in the manufacture, use, offer for sale, sale, and/or importation of DRL's ANDA product, with its proposed prescribing information, immediately and imminently upon approval of ANDA No. 20-4303.

29. Upon information and belief, immediately upon approval of ANDA No. 20-4303, DRL will infringe the '384 patent by making, using, offering to sell, selling, and/or importing DRL's ANDA product in the United States under 35 U.S.C. §§ 271(a) and/or (g), and/or by actively inducing and/or contributing to infringement by others under 35 U.S.C. §§ 271(b) and/or

(c), unless this Court orders that the effective date of any FDA approval of ANDA No. 20-4303 shall be no earlier than the expiration date of the '384 patent.

30. DRL has knowledge of the '384 patent; DRL's ANDA product constitutes a material part of at least one or more claims of the '384 patent; DRL knows that its product is especially made or adapted for use in a manner infringing at least one or more claims of the '384 patent; and DRL's ANDA product is not a staple article or commodity of commerce suitable for substantial non-infringing use.

31. The offering to sell, sale, and/or importation of DRL's ANDA product would contributorily infringe one or more claims of the '384 patent.

32. DRL has knowledge of the '384 patent and specifically intends to encourage infringement by providing prescribing information and/or through other labeling and promotional activities for DRL's ANDA product, and knows that its prescribing information and/or other labeling and promotional activities for DRL's ANDA product will induce use of DRL's ANDA product by doctors and/or patients in a manner that directly infringes of one or more claims of the '384 patent.

33. The offering to sell, sale, and/or importation of DRL's ANDA product would actively induce infringement of one or more claims of the '384 patent.

34. Unless DRL is enjoined from infringing the '384 patent, actively inducing infringement of the '384 patent, and/or contributing to the infringement by others of the '384 patent, Plaintiff will be substantially and irreparably harmed. Plaintiff has no adequate remedy at law.

COUNT II
INFRINGEMENT OF U.S. PATENT NO. 7,022,330

35. Plaintiff re-alleges and incorporates by reference paragraphs 1–34, above.

36. Bristol-Myers Squibb is the owner by assignment of the '330 patent and has the right to sue for infringement thereof. A true and correct copy of the '330 patent is attached as Exhibit B.

37. DRL's ANDA product, or use thereof, would directly infringe one or more of the claims of the '330 patent.

38. DRL's submission of ANDA No. 20-4303 seeking approval for the commercial manufacture, use, offer for sale, and/or sale of DRL's ANDA product before the expiration of the '330 patent constitutes an act of infringement of one or more claims of the '330 patent under 35 U.S.C. § 271(e)(2)(A).

39. The commercial manufacture, use, offer for sale, sale and/or importation of DRL's ANDA product would infringe and/or contribute to and/or induce infringement of one or more claims of the '330 patent.

40. Upon information and belief, DRL intends to engage in the manufacture, use, offer for sale, sale, and/or importation of DRL's ANDA product, with its proposed prescribing information, immediately and imminently upon approval of ANDA No. 20-4303.

41. Upon information and belief, immediately upon approval of ANDA No. 20-4303, DRL will infringe the '330 patent by making, using, offering to sell, selling, and/or importing DRL's ANDA product in the United States under 35 U.S.C. §§ 271(a) and/or (g), and/or by actively inducing and contributing to infringement by others under 35 U.S.C. §§ 271(b) and/or (c), unless this Court orders that the effective date of any FDA approval of ANDA No. 20-4303 shall be no earlier than the expiration date of the '330 patent.

42. DRL has knowledge of the '330 patent; DRL's ANDA product constitutes a material part of at least one or more claims of the '330 patent; DRL knows that its product is

especially made or adapted for use in a manner infringing at least one or more claims of the '330 patent; and DRL's ANDA product is not a staple article or commodity of commerce suitable for substantial non-infringing use.

43. The offering to sell, sale, and/or importation of DRL's ANDA product would contributorily infringe one or more claims of the '330 patent.

44. DRL has knowledge of the '330 patent and specifically intends to encourage infringement by providing prescribing information and/or through other labeling and promotional activities for DRL's ANDA product, and knows that its prescribing information and/or other labeling and promotional activities for DRL's ANDA product will induce use of DRL's ANDA product by doctors and/or patients in a manner that directly infringes of one or more claims of the '330 patent.

45. The offering to sell, sale, and/or importation of DRL's ANDA product would actively induce infringement of one or more claims of the '330 patent.

46. Unless DRL is enjoined from infringing the '330 patent, actively inducing infringement of the '330 patent, and/or contributing to the infringement by others of the '330 patent, Plaintiff will be substantially and irreparably harmed. Plaintiff has no adequate remedy at law.

COUNT III
INFRINGEMENT OF U.S. PATENT NO. RE41,393

47. Plaintiff re-alleges and incorporates by reference paragraphs 1–46, above.

48. Bristol-Myers Squibb is the owner by assignment of the '393 patent and has the right to sue for infringement thereof. A true and correct copy of the '393 patent is attached as Exhibit C.

49. Use of DRL's ANDA product would directly infringe one or more of the claims of the '393 patent.

50. DRL's submission of ANDA No. 20-4303 seeking approval for the commercial manufacture, use, offer for sale, and/or sale of DRL's ANDA product before the expiration of the '393 patent constitutes an act of infringement of one or more claims of the '393 patent under 35 U.S.C. § 271(e)(2)(A).

51. The commercial manufacture, use, offer for sale, sale and/or importation of DRL's ANDA product would infringe and/or contribute to and/or induce infringement of one or more claims of the '393 patent.

52. Upon information and belief, DRL intends to engage in the manufacture, use, offer for sale, sale, and/or importation of DRL's ANDA product, with its proposed prescribing information, immediately and imminently upon approval of ANDA No. 20-4303.

53. Upon information and belief, immediately upon approval of ANDA No. 20-4303, DRL will infringe the '393 patent by actively inducing and contributing to infringement by others under 35 U.S.C. §§ 271(b) and/or (c), unless this Court orders that the effective date of any FDA approval of ANDA No. 20-4303 shall be no earlier than the expiration date of the '393 patent.

54. DRL has knowledge of the '393 patent; DRL's ANDA product constitutes a material part of at least one or more claims of the '393 patent; DRL knows that its product is especially made or adapted for use in a manner infringing at least one or more claims of the '393 patent; and DRL's ANDA product is not a staple article or commodity of commerce suitable for substantial non-infringing use.

55. The offering to sell, sale, and/or importation of DRL's ANDA product would contributorily infringe one or more claims of the '393 patent.

56. DRL has knowledge of the '393 patent and specifically intends to encourage infringement by providing prescribing information and/or through other labeling and promotional activities for DRL's ANDA product, and knows that its prescribing information and/or other labeling and promotional activities for DRL's ANDA product will induce use of DRL's ANDA product by doctors and/or patients in a manner that directly infringes of one or more claims of the '393 patent.

57. The offering to sell, sale, and/or importation of DRL's ANDA product would actively induce infringement of one or more claims of the '393 patent.

58. Unless DRL is enjoined from actively inducing infringement of the '393 patent, and/or contributing to the infringement by others of the '393 patent, Plaintiff will be substantially and irreparably harmed. Plaintiff has no adequate remedy at law.

PRAYER FOR RELIEF

WHEREFORE, Plaintiff respectfully requests the following relief:

(1) a judgment that, under 35 U.S.C. § 271(e)(2)(A), DRL's submission to the FDA of ANDA No. 20-4303 to obtain approval for the commercial manufacture, use, offer for sale, or sale in, or importation into, the United States of DRL's ANDA product before the expiration of the '384 patent was an act of infringement of one or more claims of the '384 patent;

(2) a declaratory judgment that, under 35 U.S.C. § 271(a), (b), (c), and /or (g), the commercial manufacture, use, offer for sale, or sale in, or importation into, the United States of DRL's ANDA product before the expiration of the '384 patent will be an act of infringement of one or more claims of the '384 patent;

(3) a judgment that, under 35 U.S.C. §§ 271(e)(2)(A), DRL's submission to the FDA of ANDA No. 20-4303 to obtain approval for the commercial manufacture, use, offer for sale, or sale in, or importation into, the United States of DRL's ANDA product before the expiration of the '330 patent was an act of infringement of one or more claims of the '330 patent;

(4) a declaratory judgment that, under 35 U.S.C. §§ 271(a), (b), (c), and /or (g), the commercial manufacture, use, offer for sale, or sale in, or importation into, the United States of DRL's ANDA product before the expiration of the '330 patent will be an act of infringement of one or more claims of the '330 patent;

(5) a judgment that, under 35 U.S.C. § 271(e)(2)(A), DRL's submission to the FDA of ANDA No. 20-4303 to obtain approval for the commercial manufacture, use, offer for sale, or sale in, or importation into, the United States of DRL's ANDA product before the expiration of the '393 patent was an act of infringement of one or more claims of the '393 patent;

(6) a declaratory judgment that, under 35 U.S.C. §§ 271 (b) and/or (c), the commercial manufacture, use, offer for sale, or sale in, or importation into, the United States of DRL's ANDA product before the expiration of the '393 patent will be an act of infringement of one or more claims of the '393 patent;

(7) an order that the effective date of any FDA approval of DRL's ANDA product shall be no earlier than the expiration of the '384 patent, and any pediatric or other extensions thereof, in accordance with 35 U.S.C. § 271(e)(4)(A);

(8) an order that the effective date of any FDA approval of DRL's ANDA product shall be no earlier than the expiration of the'330 patent, and any pediatric or other extensions thereof, in accordance with 35 U.S.C. § 271(e)(4)(A);

(9) an order that the effective date of any FDA approval of DRL's ANDA product shall be no earlier than the expiration of the'393 patent, and any pediatric or other extensions thereof, in accordance with 35 U.S.C. § 271(e)(4)(A);

(10) a permanent injunction enjoining DRL, its affiliates and subsidiaries, and all persons and entities acting in concert with DRL from commercially manufacturing, using, offering for sale, or selling DRL's ANDA product within the United States, or importing DRL's ANDA product into the United States, until the expiration of the '384 patent, in accordance with 35 U.S.C. § 271(e)(4)(B);

(11) a permanent injunction enjoining DRL, its affiliates and subsidiaries, and all persons and entities acting in concert with DRL from commercially manufacturing, using, offering for sale, or selling DRL's ANDA product within the United States, or importing DRL's ANDA product into the United States, until the expiration of the '330 patent, in accordance with 35 U.S.C. § 271(e)(4)(B);

(12) a permanent injunction enjoining DRL, its affiliates and subsidiaries, and all persons and entities acting in concert with DRL from commercially manufacturing, using, offering for sale, or selling DRL's ANDA product within the United States, or importing DRL's ANDA product into the United States, until the expiration of the '393 patent, in accordance with 35 U.S.C. § 271(e)(4)(B);

(13) an award of damages or other relief if DRL engages in the commercial manufacture, use, offer to sell, sale, marketing, distribution, and/or importation of DRL's ANDA product, or any product that infringes the '384 patent, prior to the expiration of the '384 patent, in accordance with 35 U.S.C. § 271(e)(4)(C);

(14) an award of damages or other relief if DRL engages in the commercial manufacture, use, offer to sell, sale, marketing, distribution, and/or importation of DRL's ANDA product, or any product that infringes the '330 patent, prior to the expiration of the '330 patent, in accordance with 35 U.S.C. § 271(e)(4)(C);

(15) an award of damages or other relief if DRL engages in the commercial manufacture, use, offer to sell, sale, marketing, distribution, and/or importation of DRL's ANDA product, or any product that infringes the '393 patent, prior to the expiration of the '393 patent, in accordance with 35 U.S.C. § 271(e)(4)(C);

(16) a declaration that this is an exceptional case, and an award of attorneys' fees to Plaintiff, in accordance with 35 U.S.C. § 285;

(17) an award to Plaintiff of their costs and expenses in this action; and

(18) such further and additional relief as this Court deems just and proper.

Dated: December 21, 2012

Respectfully submitted,

By: s/Douglas S. Eakeley

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Exhibit A

US006670384B2

(12) **United States Patent**
Bandyopadhyay et al.

(10) **Patent No.: US 6,670,384 B2**
(45) **Date of Patent: Dec. 30, 2003**

(54) **METHODS OF ADMINISTERING EPOTHILONE ANALOGS FOR THE TREATMENT OF CANCER**

(75) Inventors: **Rebanta Bandyopadhyay**, Portage, MI (US); **Timothy M. Malloy**, Yardley, PA (US); **Andrea Panaggio**, West Windsor, NJ (US); **Krishnaswamy Srinivas Raghavan**, Cranbury, NJ (US); **Sailesh Amilal Varia**, Princeton Junction, NJ (US)

(73) Assignee: **Bristol-Myers Squibb Company**, Princeton, NJ (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **10/055,653**

(22) Filed: **Jan. 23, 2002**

(65) **Prior Publication Data**

US 2002/0169190 A1 Nov. 14, 2002

Related U.S. Application Data

(60) Provisional application No. 60/264,228, filed on Jan. 25, 2001, and provisional application No. 60/290,008, filed on May 11, 2001.

(51) **Int. Cl.**[7] **A61K 31/365**

(52) **U.S. Cl.** **514/365**; 514/183

(58) **Field of Search** 514/365, 183

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Primary Examiner—James H Reamer
(74) *Attorney, Agent, or Firm*—Rena Patel

(57) **ABSTRACT**

A process for formulating certain epothilone analogs for parenteral administration is disclosed wherein the analog is dissolved in a mixture of at least 50% by volume tertiary-butanol in water, the mixture is lyophilized, the resulting lyophilized product is packaged in one vial with a sufficient amount of solvent comprising anhydrous ethanol and a suitable nonionic surfactant in a second vial. All steps are carried out with protection from light. In use, the contents of the second or diluent vial are added to the lyophilized product and mixed to constitute the epothilone analog and the resulting solution is diluted with a suitable diluent to produce a solution for intravenous injection containing the epothilone analog in a concentration of from about 0.1 mg/mL to about 0.9 mg/mL. A preferred surfactant is polyethoxylated castor oil and a preferred diluent is Lactated Ringer's Injection.

105 Claims, No Drawings

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METHODS OF ADMINISTERING EPOTHILONE ANALOGS FOR THE TREATMENT OF CANCER

CROSS-REFERENCE TO RELATED APPLICATIONS

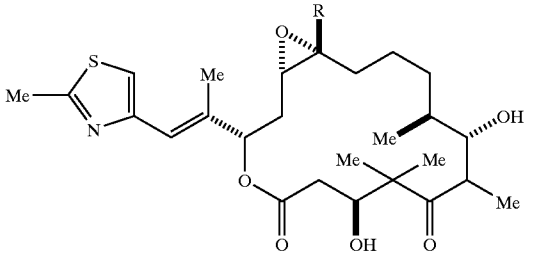
This application claims priority from provisional application serial No. 60/264,228, filed Jan. 25, 2001 and 60/290,008, filed May 11, 2001, both of which are incorporated herein by reference in their entirety.

FIELD OF THE INVENTION

The present invention relates to methods of administration for parenteral and oral compositions of certain epothilone analogs that are characterized by enhanced clinical efficacy.

BACKGROUND OF THE INVENTION

Epothilones are macrolide compounds having utility in the pharmaceutical field. For example, Epothilones A and B having the structures:

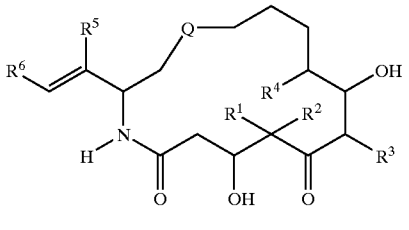


Epothilone A R = H
Epothilone B R = Me

may be found to exert microtubule-stabilizing effects similar to paclitaxel (TAXOL®) and hence cytotoxic activity against rapidly proliferating cells, such as, tumor cells or other hyperproliferative cellular disease, see Hofle et al., *Angew. Chem. Int. Ed. Engl.*, Vol. 35, No.13/14, 1567–1569 (1996); WO 93/10121 published May 27, 1993; and WO 97/19086 published May 29, 1997.

Derivatives and analogs of Epothilones A and B have been synthesized and may be used to treat a variety of cancers and other abnormal proliferative diseases. Such analogs are disclosed in Hofle et al., Id.; Nicolaou et al., *Angew Chem. Int. Ed. Engl.*, Vol. 36, No. 19, 2097–2103 (1997); and Su et al., *Angew Chem. Int. Ed. Engl.*, Vol. 36, No. 19, 2093–2097 (1997).

Analogs of the epothilones that have been found to have advantageous activity are represented by formula I:



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wherein the various symbols are as defined below. While these compounds possess significant therapeutic properties, they also present difficulties to those skilled in the art of pharmaceutical compounding, as a result of certain

properties, as will be detailed hereinbelow. In accordance with the present invention, a formulation has been found whereby the epothilone analogs described above can be safely dispensed and administered via injection, without appreciable loss of potency.

Furthermore, many anti-cancer drugs have toxicity concerns. Indeed, the therapeutic profile of many potent antitumor drugs is poor as a result of toxicity. Therefore, there is also a need for methods of administration and dosing schedules that reduce or avoid the toxicity associated with antitumor agents. The methods can allow exploitation of potent antitumor agents that would otherwise not be used clinically.

SUMMARY OF THE INVENTION

The invention encompasses a novel dosing schedule for epothilone compounds, which schedule is useful in treating patients having solid tumors, particularly advanced solid tumors. Further, the methods of the invention can be used to treat and/or prevent metastatic as well as primary tumors. In one embodiment, the invention encompasses the treatment of patients that have previously received either or both radiation therapy and chemotherapy for solid tumors. It has also been found that the epothilone compounds of the invention particularly the preferred compound, [1S-[1R*, 3R*(E),7R*,10S*,11R*,12R*,16S*]]-7,11-Dihydroxy-8,8,10,12,16-pentamethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl) ethenyl]-4-aza-17-oxabicyclo[14.1.0]heptadecane-5,9-dione, can be used to treat tumors refactory to radiation therapy or chemotherapy. The methods of the invention are useful against cancer cells, and thus, tumors, that are naturally or become insensitive to paclitaxel.

In one embodiment, the dosing schedule of the invention comprises the weekly administration of an epothilone compound of the invention preferably as a one (1) hour infusion weekly on a continuous basis. In another embodiment, the administration is made weekly for a three week cycle. The dose range for weekly infusion is from 1 mg/m^2 to 30 mg/m^2 and more preferably 1 mg/m^2 to 25 mg/m^2. In another embodiment, the dosing schedule includes both oral and intravenous administration of the same epothilone compound. For example, the weekly infusion can be followed or preceded by an oral administration of 20 mg/m^2 or greater. In a specific embodiment, the administration regimen includes a three (3) week cycle of intravenous infusion once per week for about one (1) hour followed by or preceded by an oral dose administered one or more times in the week before the first intravenous administration of a cycle or the week after the last intravenous administration of a cycle. Other protocols are also encompassed within the present invention including but not limited to:

(a) a daily dosing for 5 to 10 days followed by at least 3 days of no dosing;

(b) weekly dosing for two to ten weeks followed by at least one week of no dosing; and

(c) dosing once every three weeks followed by at least one week of no dosing.

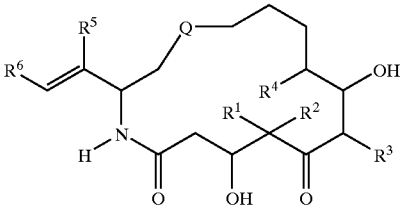
The invention also contemplates the use of H_1 and H_2 antihistamines before, after and/or before and after a cycle of epothilone administration. Similarly, the invention encompasses the use of other chemotherapeutics, particularly anti-tumor agents, with epothilone cycle alone, or with the H_1 and H_2 blockers and the epothilones.

In another embodiment, the epothilone dosing schedule is used after the standard regimen of paclitaxel.

As discussed herein a wide variety of cancers are encompassed by the methods of the present invention. In a pre-

ferred embodiment, the methods of the invention are for the treatment of solid tumors including but not limited to breast, head and neck, sarcoma, colorectal, UPT, melanoma, oesophagus, renal, cervix, thyroid, anal, ovarian, and colon.

The methods and compositions of the present invention describes a formulation and the preparation thereof for epothilone analogs represented by formula I:



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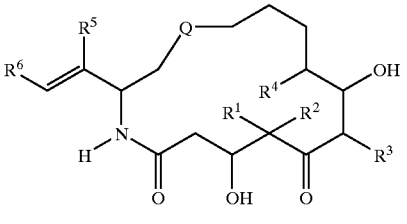
wherein the various symbols are as defined below.

In one embodiment of the formulations of the present invention, the epothilone analog is initially solubilized with a mixture of tertiary-butanol and water and then lyophilized under optimized conditions. The lyophilized drug is reconstituted first with a mixture of a polyethoxylated castor oil surfactant and anhydrous ethanol, and thereafter diluted with Lactated Ringer's Injection to a concentration appropriate for administration.

DETAILED DESCRIPTION OF THE INVENTION

In an embodiment, the present invention provides an advantageous formulation for the administration of epothilone analogs represented by formula I:



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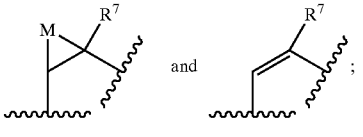
As used in formula I and throughout the specification, Q is selected from the group consisting of:



and

;

M is selected from the group consisting of oxygen, sulfur, NR^8, and CR^9R^{10};

each R^1, R^2, R^3, R^4, R^5, R^7, R^{11}, R^{12}, R^{13} and R^{14} is, independently, selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, substituted aryl and heterocyclo, and wherein R^1 and R^2 are alkyl, they can be joined to form cycloalkyl;

R^6 is selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, cycloalkyl, heterocyclo and substituted heterocyclo;

R^8 is selected from the group consisting of hydrogen, alkyl, substituted alkyl, $R^{11}C{=}O$, $R^{12}OC{=}O$ and $R^{13}SO_2$; and

each R^9 and R^{10} is, independently, selected from the group consisting of hydrogen, halogen, alkyl, substituted alkyl, aryl, heterocyclo, hydroxy, $R^{14}C{=}O$, and $R^{15}OC{=}O$.

The following are definitions of various terms used herein to describe the present invention. These definitions apply to the terms as they are used throughout this specification, unless otherwise limited in specific instances, either individually or as part of a larger group.

The term "alkyl" refers to optionally substituted straight- or branched-chain saturated hydrocarbon groups having from 1 to about 20 carbon atoms, preferably from 1 to about 7 carbon atoms. The expression "lower alkyl" refers to optionally substituted alkyl groups having from 1 to about 4 carbon atoms.

The term "substituted alkyl" refers to an alkyl group substituted by, for example, one to four substituents, such as, halo, trifluoromethyl, trifluoromethoxy, hydroxy, alkoxy, cycloalkyoxy, heterocylooxy, oxo, alkanoyl, aryl, aryloxy, aralkyl, alkanoyloxy, amino, alkylamino, arylamino, aralkylamino, cycloalkylamino, heterocycloamino, disubstituted amino in which the two substituents on the amino group are selected from alkyl, aryl, aralkyl, alkanoylamino, aroylamino, aralkanoylamino, substituted alkanoylamino, substituted arylamino, substituted aralkanoylamino, thiol, alkylthio, arylthio, aralkylthio, cycloalkylthio, heterocyclothio, alkylthiono, arylthiono, aralkylthiono, alkylsulfonyl, arylsulfonyl, aralkylsulfonyl, sulfonamido (e.g., SO_2NH_2), substituted sulfonamido, nitro, cyano, carboxy, carbamyl (e.g., $CONH_2$), substituted carbamyl (e.g., CONH alkyl, CONH aryl, CONH aralkyl or instances where there are two substituents on the nitrogen selected from alkyl, aryl or aralkyl), alkoxycarbonyl, aryl, substituted aryl, guanidino and heterocyclos, such as, indolyl, imidazolyl, furyl, thienyl, thiazolyl, pyrrolidyl, pyridyl, pyrimidyl and the like. Wherein, as noted above, the substituents themselves are further substituted, such further substituents are selected from the group consisting of halogen, alkyl, alkoxy, aryl and aralkyl. The definitions given herein for alkyl and substituted alkyl apply as well to the alkyl portion of alkoxy groups.

The term "halogen" or "halo" refers to fluorine, chlorine, bromine and iodine.

The term "ring system" refers to an optionally substituted ring system containing one to three rings and at least one carbon to carbon double bond in at least one ring. Exemplary ring systems include, but are not limited to, an aryl or a partially or fully unsaturated heterocyclic ring system, which may be optionally substituted.

The term "aryl" refers to monocyclic or bicyclic aromatic hydrocarbon groups having from about 6 to about 12 carbon atoms in the ring portion, for example, phenyl, naphthyl, biphenyl and diphenyl groups, each of which may be substituted.

The term "aralkyl" refers to an aryl group bonded to a larger entity through an alkyl group, for example, a benzyl group.

The term "substituted aryl" refers to an aryl group substituted by, for example, one to four substituents such as alkyl; substituted alkyl, halo, trifluoromethyl, trifluoromethoxy, hydroxy, alkoxy, cycloalkyloxy, heterocyclooxy, alkanoyl, alkanoyloxy, amino, alkylamino, dialkylamino, aralkylamino, cycloalkylamino, heterocycloamino, alkanoylamino, thiol, alkylthio, cycloalkylthio, heterocyclothio, ureido, nitro, cyano, carboxy, carboxyalkyl, carbamyl, alkoxycarbonyl, alkylthiono, arylthiono, alkysulfonyl, sulfonamido, aryloxy

and the like. The substituent may be further substituted by one or more members selected from the group consisting of halo, hydroxy, alkyl, alkoxy, aryl, substituted alkyl, substituted aryl and aralkyl.

The term "cycloalkyl" refers to optionally substituted saturated cyclic hydrocarbon ring systems, preferably containing 1 to 3 rings and 3 to 7 carbons per ring, which may be further fused with an unsaturated C_3–C_7 carbocyclic ring. Exemplary groups include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, cyclodecyl, cyclododecyl, and adamantyl. Exemplary substituents include one or more alkyl groups as described above, or one or more of the groups described above as substituents for alkyl groups.

The terms "heterocycle", "heterocyclic" and "heterocyclo" refer to an optionally substituted, unsaturated, partially saturated, or fully saturated, aromatic or nonaromatic cyclic group, for example, which is a 4 to 7 membered monocyclic, 7 to 11 membered bicyclic, or 10 to 15 membered tricyclic ring system, which has at least one heteroatom in at least one carbon atom-containing ring. Each ring of the heterocyclic group containing a heteroatom may have 1, 2 or 3 heteroatoms selected from nitrogen atoms, oxygen atoms and sulfur atoms, where the nitrogen and sulfur heteroatoms may also optionally be oxidized and the nitrogen heteroatoms may also optionally be quaternized. The heterocyclic group may be attached at any heteroatom or carbon atom.

Exemplary monocyclic heterocyclic groups include pyrrolidinyl, pyrrolyl, indolyl, pyrazolyl, oxetanyl, pyrazolinyl, imidazolyl, imidazolinyl, imidazolidinyl, oxazolyl, oxazolidinyl, isoxazolinyl, isoxazolyl, thiazolyl, thiadiazolyl, thiazolidinyl, isothiazolyl, isothiazolidinyl, furyl, tetrahydrofuryl, thienyl, oxadiazolyl, piperidinyl, piperazinyl, 2-oxopiperazinyl, 2-oxopiperidinyl, 2-oxopyrrolidinyl, 2-oxazepinyl, azepinyl, 4-piperidonyl, pyridyl, N-oxo-pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, tetrahydropyranyl, tetrahydrothiopyranyl, tetrahydrothiopyranyl sulfone, morpholinyl, thiomorpholinyl, thiomorpholinyl sulfoxide, thiomorpholinyl sulfone, 1,3-dioxolane and tetrahydro-1, 1-dioxothienyl, dioxanyl, isothiazolidinyl, thietanyl, thiiranyl, triazinyl, and triazolyl, and the like.

Exemplary bicyclic heterocyclic groups include benzothiazolyl, benzoxazolyl, benzothienyl, quinuclidinyl, quinolinyl, quinolinyl-N-oxide, tetrahydroisoquinolinyl, isoquinolinyl, benzimidazolyl, benzopyranyl, indolizinyl, benzofuryl, chromonyl, coumarinyl, cinnolinyl, quinoxalinyl, indazolyl, pyrrolopyridyl, furopyridinyl (such as furo[2,3-c]pyridinyl, furo[3,1-b]pyridinyl] or furo[2,3-b] pyridinyl), dihydroisoindolyl, dihydroquinazolinyl (such as 3,4-dihydro-4-oxo-quinazolinyl), benzisothiazolyl, benzisoxazolyl, benzodiazinyl, benzofurazanyl, benzothiopyranyl, benzotriazolyl, benzpyrazolyl, dihydrobenzofuryl, dihydrobenzothienyl, dihydrobenzothiopyranyl, dihydrobenzothiopyranyl sulfone, dihydrobenzopyranyl, indolinyl, isochromanyl, isoindolinyl, naphthyridinyl, phthalazinyl, piperonyl, purinyl, pyridopyridyl, quinazolinyl, tetrahydroquinolinyl, thienofuryl, thienopyridyl, thienothienyl, and the like.

Exemplary substituents for the terms "ring system," "heterocycle," "heterocyclic," and "heterocyclo" include one or more substituent groups as described above for substituted alkyl or substituted aryl, and smaller heterocyclos, such as, epoxides, aziridines and the like.

The term "alkanoyl" refers to —C(O)-alkyl.

The term "substituted alkanoyl" refers to —C(O)-substituted alkyl.

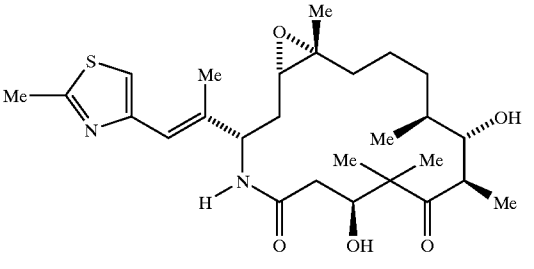
The term "heteroatoms" shall include oxygen, sulfur and nitrogen.

The compounds represented by formula I form salts with a variety of organic and inorganic acids. Such salts include those formed with hydrogen chloride, hydrogen bromide, methanesulfonic acid, hydroxyethanesulfonic acid, sulfuric acid, acetic acid, trifluoroacetic acid, maleic acid, benzenesulfonic acid, toluenesulfonic acid and various others as are recognized by those of ordinary skill in the art of pharmaceutical compounding. Such salts are formed by reacting a compound represented by formula I in an equivalent amount of the acid in a medium in which the salt precipitates or in an aqueous medium followed by evaporation.

In addition, zwitterions ("inner salts") can be formed and are included within the term salts as used herein.

A particularly preferred epothilone analog within those represented by formula I is [1S-[1R*,3R*(E),7R*,10S*, 11R*, 12R*,16S*,]]-7,11-Dihydroxy-8,8,10,12,16-pentamethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-4-aza-7-oxabicyclo[14.1.0]heptadecane-5,9-dione represented by formula II:

II



The compounds represented by formulae I and II above, also referred to herein as "the epothilone compounds of the invention," and their preparation are described in U.S. patent application Ser. No. 09/170,582, filed Oct. 13, 1998, and U.S. patent application Ser. No. 09/280,191, filed Mar. 29, 1999, the disclosure of which is incorporated herein by reference. The compounds represented by formulae I and II above may exist as multiple optical, geometric, and stereoisomers. While the compounds shown herein are depicted for one optical orientation, included within the present invention are all isomers and mixtures thereof.

The compounds represented by formulae I and II above are microtubule-stabilizing agents. They are thus useful in the treatment of a variety of cancers and other proliferative diseases including, but not limited to, the following:

- carcinoma, including that of the bladder, breast, colon, kidney, liver, lung, ovary, pancreas, stomach, cervix, thyroid and skin, including squamous cell carcinoma;
- hematopoietic tumors of lymphoid lineage, including leukemia, acute lymphocytic leukemia, acute lymphoblastic leukemia, B-cell lymphoma, T-cell lymphoma, Hodgkins lymphoma, non-Hodgkins lymphoma, hairy cell lymphoma and Burketts lymphoma;
- hematopoietic tumors of myeloid lineage, including acute and chronic myelogenous leukemias and promyelocytic leukemia;
- tumors of mesenchymal origin, including fibrosarcoma and rhabdomyoscarcoma;
- other tumors, including melanoma, seminoma, teratocarcinoma, neuroblastoma and glioma;
- tumors of the central and peripheral nervous system, including astrocytoma, neuroblastoma, glioma, and schwannomas;
- tumors of mesenchymal origin, including fibrosarcoma, rhabdomyoscaroma, and osteosarcoma; and

other tumors, including melanoma, xeroderma pigmentosum, keratoacanthoma, seminoma, thyroid follicular cancer and teratocarcinoma.

The compounds represented by formulae I and II are useful for treating patients who have been previously treated for cancer, as well as those who have not previously been treated for cancer. Indeed, the methods and compositions of this invention can be used in first-line and second-line cancer treatments. Furthermore, the compounds represented by formulae I and II are useful for treating refractory cancers.

The compounds represented by formulae I and II above will also inhibit angiogenesis, thereby affecting the growth of tumors and providing treatment of tumors and tumor-related disorders. Such anti-angiogenesis properties of the compounds represented by formulae I and II will also be useful in the treatment of other conditions responsive to anti-angiogenesis agents including, but not limited to, certain forms of blindness related to retinal vascularization, arthritis, especially inflammatory arthritis, multiple sclerosis, restinosis and psoriasis.

Compounds represented by formulae I and II will induce or inhibit apoptosis, a physiological cell death process critical for normal development and homeostasis. Alterations of apoptotic pathways contribute to the pathogenesis of a variety of human diseases. Compounds represented by formulae I and II, as modulators of apoptosis, will be useful in the treatment of a variety of human diseases with aberrations in apoptosis including, but not limited to, cancer and precancerous lesions, immune response related diseases, viral infections, degenerative diseases of the musculoskeletal system and kidney disease.

Each of the compounds represented by formulae I and II may also be formulated or co-administered with other therapeutic agents that are selected for their particular usefulness in administering therapies associates with the aforementioned conditions. For example, each of the compounds of formulae I and II may be formulated with agents to prevent nausea, hypersensitivity, and gastric irritation, such as antiemetics, and H_1 and H_2 antihistamines. The above therapeutic agents, when employed in combination with the compound of formulae I or II, may be used in those amounts indicated in the Physicians' Desk Reference (PDR) or as otherwise determined by one of ordinary skill in the art.

Furthermore, compounds of formulae I or II may be administered in combination with other anti-cancer and cytotoxic agents and treatments useful in the treatment of cancer or other proliferative diseases. Especially useful are anti-cancer and cytotoxic drug combinations wherein the second drug chosen acts in a different manner or different phase of the cell cycle, e.g., S phase, than the present compounds of formula I and II which exert their effects at the G_2-M phase. Example classes of anti-cancer and cytotoxic agents include, but are not limited to, alkylating agents, such as nitorgen mustards, alkyl sulfonates, nitrosoureas, ethylenimines, and triazenes; antimetabolites, such as folate antagonists, purine analogues, and pyrimidine analogues; antibiotics, such as anthracyclines, bleomycins, mitomycin, dactinomycin, and plicamycin; enzymes, such as L-asparaginase; farnesyl-protein transferase inhibitors; hormonal agents, such as glucocorticoids, estrogens/antiestrogens, androgens/antiandrogens, progestins, and luteinizing hormone-releasing hormone anatagonists, octreotide acetate; microtubule-disruptor agents, such as ecteinascidins or their analogs and derivatives; microtubule-stabilizing agents such as paclitaxel (Taxol®), docetaxel (Taxotere®); plant-derived products, such as vinca alkaloids, epipodophyllotoxins, taxanes; and topoisomerase inhibitors; prenyl-protein transferase inhibitors; and miscellaneous agents such as, hydroxyurea, procarbazine, mitotane, hexamethylmelamine, platinum coordination complexes such as cisplatin and carboplatin; and other agents used as anti-cancer and cytotoxic agents such as biological response modifiers, growth factors; immune modulators, and monoclonal antibodies. Compounds represented by formulae I and II may also be used in conjunction with radiation therapy.

Representative examples of these classes of anti-cancer and cytotoxic agents include, but are not limited to, mechlorethamine hydrochlordie, cyclophosphamide, chlorambucil, melphalan, ifosfamide, busulfan, carmustin, lomustine, semustine, streptozocin, thiotepa, dacarbazine, methotrexate, thioguanine, mercaptopurine, fludarabine, pentastatin, cladribin, cytarabine, fluorouracil, doxorubicin hydrochloride, daunorubicin, idarubicin, bleomycin sulfate, mitomycin C, actinomycin D, safracins, saframycins, quinocarcins, discodermolides, vincristine, vinblastine, vinorelbine tartrate, etoposide, teniposide, paclitaxel, tamoxifen, estramustine, estramustine phosphate sodium, flutamide, buserelin, leuprolide, pteridines, diyneses, levamisole, aflacon, interferon, interleukins, aldesleukin, filgrastim, sargramostim, rituximab, BCG, tretinoin, irinotecan hydrochloride, betamethosone, gemcitabine hydrochloride, altretamine, and topoteca and any analogs or derivatives thereof.

Preferred members of these classes include, but are not limited to, paclitaxel, cisplatin, carboplatin, doxorubicin, carminomycin, daunorubicin, aminopterin, methotrexate, methopterin, mitomycin C, ecteinascidin 743, porfiromycin, 5-fluorouracil, 6-mercaptopurine, gemcitabine, cytosine arabinoside, podophyllotoxin or podophyllotoxin derivatives such as etoposide, etoposide phosphate or teniposide, melphalan, vinblastine, vincristine, leurosidine, vindesine, and leurosine.

Examples of anti-cancer and other cytotoxic agents include the following: cyclin dependent kinase inhibitors as found in WO 99/24416; and prenyl-protein transferase inhibitors as found in WO 97/30992 and WO 98/54966.

The compounds may also be administered with or after anti-cancer and cytotoxic agents that are neurotoxic, i.e., poisonous to the nervous system.

Without being bound by any theory regarding mechanism or morphology, the compounds represented by formulae I and II may also be used to treat conditions other than cancer or other proliferative diseases. Such conditions include, but are not limited to viral infections such as herpesvirus, poxvirus, Epstein-Barr virus, Sindbis virus and adenovirus; autoimmune diseases such as systemic lupus erythematosus, immune mediated glomerulonephritis, rheumatoid arthritis, psoriasis, inflammatory bowel diseases and autoimmune diabetes mellitus; neurodegenerative disorders such as Alzeimer's disease, AIDS-related dementia, Parkinson's disease, amyotrophic lateral sclerosis, retinitis pigmentosa, spinal muscular atrophy and cerebellar degeneration; AIDS; myelodysplastic syndromes; aplastic anemia; ischemic injury associated myocardial infarctions; stroke and reperfusion injury; restenosis; arrhythmia; atherosclerosis; toxin-induced or alcohol induced liver diseases; hematological diseases such as chronic anemia and aplastic anemia; degenerative diseases of the musculoskeletal system such as osteoporosis and arthritis; aspirin-sensitive rhinosinusitis; cystic fibrosis; multiple sclerosis; kidney diseases; and cancer pain.

The compounds represented by formulae I and II, particularly the latter, are difficult to formulate in that they

possess very low solubility in aqueous media, rapidly degrade in contact with aqueous media, are sensitive to low pH when in solution, are light sensitive, are "Class D" cytotoxic, and have exceptionally poor wetting characteristics. Any one or two of these characteristics might be compensated for in compounding a pharmaceutical formulation for intravenous administration, but the combination of all of them presents a formidable challenge to the pharmaceutical compounding chemist. Given the constraint that materials to be utilized in compounding an intravenous formulation must be approved for intravenous administration, the formulation provided in accordance with the present invention unexpectedly was found to be suitable for overcoming the properties of the subject epothilone analogs, as noted above, that make them difficult to formulate. Initially, because of the fact that the subject epothilone analogs are poorly soluble in aqueous media and, in fact, rapidly degrade in contact therewith, it was decided that they should be formulated in lyophilized form.

It has been found that a suitable media to form a solution of the subject compounds for lyophilization is a mixture of tertiary-butanol and water for injection. This mixture must be at least about 50% v/v, preferably from about 50% to about 80% v/v tertiary butanol to prevent degradation of the subject epothilone analogs. Further, due to the exceptionally poor wetting characteristics of the subject epothilone analogs, the initial solution must be effected utilizing a mixture of at least about 60% v/v, preferably from about 60% to about 95% v/v, tertiary butanol and water. Once the solution is made, the requisite amount of water or tertiary-butanol-water mixture can be added to achieve the final concentration for lyophilization as stated above.

It has unexpectedly been found that the stability of the subject epothilone analogs can be significantly enhanced by carrying out the preparation of the solution at a temperature below ambient, preferably from about 5 EC to about 15 EC, more preferably about 5 EC. Further, both the process of forming the solution and subsequent lyophilization are to be carried out in vessels such that the epothilone analogs are protected from exposure to light. It is also beneficial to carry out the lyophilization in comparatively small batches so that the epothilone analogs are exposed to an aqueous medium for a minimum amount of time.

The primary drying stage of lyophilization of the solution formed as described above is carried out at temperatures from about −10 EC to about −40 EC, preferably about −25 EC, under high vacuum, ie., from about 50 millitorr to about 300 millitorr, preferably about 200 millitorr, for an extended period, i.e., from about 24 hours to about 96 hours, preferably about 48 hours. Lyophilization in this temperature range produces an amorphous product which is desirable for an intravenous preparation. Those of ordinary skill in the art will appreciate that conventional procedures, such as powder X-ray diffraction, can be utilized to confirm the amorphous nature of the lyophilized product.

The residual solvents in the product are removed by a secondary drying stage that is carried out at comparatively low temperatures, i.e., from about 10 EC to about 30 EC, preferably about 25 EC, under high vacuum, i.e., from about 50 millitorr to about 300 millitorr, preferably about 150 millitorr for an extended period, i.e., from about 24 hours to about 96 hours, preferably about 48 hours.

It has unexpectedly been found that the stability of lyophilized epothilone analogs described herein are not enhanced by excipients commonly utilized for such purposes, such as lactose, mannitol, dextran and the like. Certain of these excipients may actually have a negative effect on the stability of the lyophilized product (lyophile). Hence, the epothilone analogs formulated in accordance with the present invention are lyophilized neat, i.e., without any excipient.

The lyophilized epothilone analogs represented by formulae I and II are reconstituted with a mixture of equal parts by volume of Dehydrated Alcohol, USP and a nonionic surfactant, preferably a polyoxyethylated castor oil surfactant available from GAF Corporation, Mount Olive, N.J., under the trademark, Cremophor EL. The lyophilized product and vehicle for reconstitution are packaged separately in appropriately light-protected vials. To minimize the amount of surfactant in the reconstituted solution, only a sufficient amount of the vehicle is provided to form a solution having a concentration of about 2 mg/mL to about 4 mg/mL of the epothilone analog. Once dissolution of the drug is achieved, the resulting solution is further diluted prior to injection with a suitable parenteral diluent. Such diluents are well known to those of ordinary skill in the art. These diluents are generally available in clinical facilities. It is, however, within the scope of the present invention to package the subject epothilone analogs with a third vial containing sufficient parenteral diluent to prepare the final concentration for administration. A preferred diluent is Lactated Ringer's Injection. The final concentration for administration would preferably contain from about 0.1 mg/mL to about 0.9 mg/mL of the epothilone analog.

The final dilution of the reconstituted epothilone analog in the formulation of the invention may be carried out with other preparations having similar utility, for example, 5% Dextrose Injection, Lactated Ringer's and Dextrose Injection, Sterile Water for Injection, and the like. However, because of its narrow pH range, pH 6.0 to 7.5, Lactated Ringer's Injection is preferred. Per 100 mL, Lactated Ringer's Injection contains Sodium Chloride USP 0.6 g, Sodium Lactate 0.31 g, Potassium chloride USP 0.03 g and Calcium Chloride-2H20 USP 0.02 g. The osmolarity is 275 mOsmol/L, which is very close to isotonicity.

The constituted preparation according to the present invention, i.e., the solution of the epothilone analog in the alcohol-surfactant vehicle, can be stored for up to about 24 hours before being further diluted for administration. It has been found that the incidence of allergic reactions encountered due to the presence of the surfactant in the formulation is minimized by keeping its concentration at the minimum necessary to effect solution of the epothilone analog. Further, the incidence of such reactions is about the same as has been experienced with other parenterally administered pharmaceuticals containing it, such as cyclosporine. This observed level of allergic reaction with the present formulation is significantly lower that has been experienced with certain other oncology agents, such as Paclitaxel.

The present invention is also directed to methods of treating cancer and other hyperproliferative diseases in patients comprising administering to the patient a therapeutically effective amount of one or more compounds represented by formulae I and II. The compounds of formula I and II may be administered intravenously or orally, preferably both orally and intravenously. Preferably, the compounds of formulae I and II are administered with one or more additional agents to prevent nausea, hypersennsitivity, or gastric irritation such as an anti-emetic or an H_1 or H_2 antihistamine.

The amount of a compound represented by formulae I and II administered by each IV infusion, or orally, or both may be determined by one of ordinary skill in the art, and includes exemplary dosage amounts for a human of from

about 0.01 mg/kg/day to about 200 mg/kg/day, which may be administered in a single dose or in the form of individual divided doses, such as from 1 to about 4 times per day. Preferably, the compounds are administered in a dosage of less than about 100 mg/kg/day, and more preferably less than about 25 mg/kg/day in a single dose or in about 2 to about 4 divided doses. It will be understood that the specific dose level and frequency of dosage for any particular subject may be varied and will depend upon a variety of factors including the activity of the specific compound employed, the metabolic stability and length of action of that compound, the species, age, body weight, general health, sex and diet of the subject, the mode and time of administration, rate of excretion, drug combination, and severity of the particular condition. Preferred subjects for treatment include animals, most preferably mammalian species such as humans, and domestic animals such as dogs, cats and the like, subject to the aforementioned disorders.

Typically the compounds of formulae I and II are administered until the patient shows a response, for example, a reduction in tumor size, or until dose limiting toxicity is reached. One or ordinary skill in the art will readily know when a patient shows a response or when dose limiting toxicity is reached. The common dose limiting toxicities associated with compounds of formulae I and II include, but are not limited to, fatigue, arthralgia/myalgia, anorexia, hypersensitivity, neutropenia, thrombocytopenia, and neurotoxicity.

When administered intravenously, the compounds of formulae I and II are preferably administered using the formulations of the invention. Generally, the compounds of Formulae I and II are administered by IV infusion over a period of from about 10 minutes to about 3 hours, preferably about 30 minutes to about 2 hours, more preferably about 45 minutes to 90 minutes, and most preferably about 1 hour. Typically, the compounds are administered intravenously in a dose of from about 0.5 mg/m^2 to 65 mg/m^2, preferably about 1 mg/m^2 to 50 mg/m^2, more preferably about 2.5 mg/m^2 to 30 mg/m^2, and most preferably about 25 mg/m^2.

One of ordinary skill in the art would readily know how to convert doses from mg/kg to mg/m2 given either or both the height and or weight of the patient (See, e.g., http://www.fda.gov/cder/cancer/animalframe.htm).

When administered orally the compounds of formulae I and II are preferably administered in combination with a pharmaceutically acceptable acid neutralizing buffer. The buffer neutralizes acid in the stomach of the patient so that the rate of decomposition of the compounds of formulae I and II is sufficiently decreased so that they remain in the gastrointestinal tract for sufficient time to be absorbed. The compounds of formulae I and II may also be administered with an anti-acid such as hydroxides of aluminum and magnesium; carbonates, such as sodium carbonate and calcium carbonate; silicates; and phosphates to neutralize the acid in the stomach before during or after administration of the compounds of formulae I and II.

As used herein, the term "pharmaceutically acceptable acid neutralizing buffer" refers to a combination of a pharmaceutically acceptable non-toxic acid and a pharmaceutically acceptable non-toxic salt of an acid that when added to a solution provides a solution that is more resistant to change of pH, compared to a solution without the buffer, when acid or alkali is added to the solution. The term "pharmaceutically acceptable acid neutralizing buffer" also includes compounds, such as basic compounds, that when added to an acidic solution neutralizes the acid and increases the pH of the solution.

In one embodiment of the invention, the compounds of formulae I and II and the pharmaceutically acceptable acid neutralizing buffer are provided in a single oral dosage form and are administered simultaneously. The single composition comprising the combination of the compounds of formulae I and II may be administered as a solid oral dosage form (e.g., a tablet, capsule, or powder) or a liquid oral dosage form (e.g., a solution, suspension, or elixir). The solution or suspension can be constituted just prior to administration using the appropriate solvents or cosolvents to dissolve the epothilone and the buffer components.

For example, the compounds of formulae I and II and the pharmaceutically acceptable acid neutralizing buffer may be orally administered simultaneously as a solution of the epothilone of formula (I) or (II) dissolved in a liquid comprising propylene glycol:ethanol:phosphate buffer (for example at 1M, about pH 8) in a ratio of about 58:12:30, respectively.

The compounds of formulae I and II and the pharmaceutically acceptable acid neutralizing buffer can also be provided as separate distinct pharmaceutical compositions and administered separately. Each of which are administered as a solid oral dosage form or a liquid oral dosage form. When the compounds of formulae I and II and the pharmaceutically acceptable acid neutralizing buffer are administered separately, the pharmaceutically acceptable acid neutralizing buffer may be orally administered before, after, or both before and after the compounds of formulae I and II is administered. Preferably, the pharmaceutically acceptable acid neutralizing buffer is administered both before and after oral administration of the compounds of formulae I and II, in an amount sufficient to neutralize the stomach acid. When the pharmaceutically acceptable acid neutralizing buffer is administered before the compounds of formulae I and II it is administered within about 5 hours preferably within about 3 hours, more preferably within about 1 hour, and most preferably with about 10 minutes before the compounds of formulae I and II is administered. When the pharmaceutically acceptable acid neutralizing buffer is administered after the compounds of formulae I and II it is administered within about 5 hours, preferably within about 3 hours, more preferably within about 1 hour, and most preferably within about 10 minutes after the compounds of formulae I and II is administered.

The compounds of formulae I and II can also be administered as an enteric coated pill or capsule to delay release of the epothilone until after the pharmaceutically effective acid neutralizing buffer is administered. Enteric coated tablets and capsules are capsules coated with a substances that resist solution in a gastric fluid but disintegrate in the intestine.

Typically, the pharmaceutically acceptable acid neutralizing buffer is administered in an amount sufficient to deliver at least about 20 milliequivalents of acid neutralization capacity, preferably at least about 30 milliequivalents of acid neutralization capacity, more preferably at least about 40 milliequivalents of acid neutralization capacity, and most preferably at least about 50 milliequivalents of acid neutralization capacity. Typically, the pharmaceutically acceptable acid neutralizing buffer is administered as an aqueous solution having a pH of between about 5 to 9, preferably about 6 to 8.5, and more preferably about 7 to 8. Any pharmaceutically acceptable acid neutralizing buffer that provides a solution having a pH in the desired range may be used in the methods of the invention. Preferably, the pharmaceutically acceptable acid neutralizing buffer is a dibasic phosphate-monobasic phosphate buffer or a dibasic phosphate buffer-citric acid-citrate buffer.

For example, oral administration of the compounds of formulae I and II can involve first orally administering to the patient the pharmaceutically acceptable acid neutralizing buffer as about 150 mL of an aqueous solution comprising anhydrous dibasic sodium phosphate (about 0.2 M), sodium citrate dihydrate (about 0.07 M), and anhydrous citric acid (about 0.008 M) at a pH of about 7.4; followed by oral administration of the compounds of formulae I and II as a liquid dosage form in a propylene glycol:ethanol system having a ratio of about 80:20; followed by oral administration of another about 150 mL aqueous solution comprising anhydrous dibasic sodium phosphate (about 0.2 M), sodium citrate dihydrate (about 0.07 M), and anhydrous citric acid (about 0.008 M) at a pH of about 7.4.

As discussed above, the compounds of formulae I and II can be administered orally, intravenously, or both. In particular, the methods of the invention encompass dosing protocols such as once a day for 2 to 10 days, preferably every 3 to 9 days, more preferably every 4 to 8 days and most preferably every 5 days. In one embodiment there is a period of 3 days to 5 weeks, preferably 4 days to 4 weeks, more preferably 5 days to 3 weeks, and most preferably 1 week to 2 weeks, in between cycles where there is no treatment. In another embodiment the compounds of formulae I or II can be administered orally, intravenously, or both, once a day for 3 days, with a period of preferably 1 week to 3 weeks in between cycles where there is no treatment. In yet another embodiment the compounds of formulae I or II can be administered orally, intravenously, or both, once a day for 5 days, with a period of preferably 1 week to 3 weeks in between cycles where there is no treatment.

In one preferred embodiment the treatment cycle for administration of the compounds of formulae I or II is once daily for 5 consecutive days and the period between treatment cycles is from 2 to 10 days, preferably one week.

The compounds of formulae I and II can also be administered orally, intravenously, or both once every 1 to 10 weeks, preferably every 2 to 8 weeks, more preferably every 3 to 6 weeks, and even more preferably every 3 weeks.

In another method of the invention, the compounds of formulae I and II are administered in a 28 day cycle wherein the compound of formulae I and II are intravenously administered on days 1, 7, and 14 and orally administered on day 21. Alternatively, the compounds of formulae I and II are administered in a 28 day cycle wherein the compound of formulae I and II are orally administered on day 1 and intravenously administered on days 7, 14, and 28.

According to the methods of the invention, the compounds of formulae I and II are administered until the patient shows a response, for example, a reduction in tumor size, or until dose limiting toxicity is reached.

Many anti-cancer agents are neurotoxic, e.g., they are known to cause side effects of the central and peripheral nervous system. This invention further encompasses the use of compounds of formulae I and II in patients previously experiencing neurotoxicity with other anti-cancer agents. Although, the compounds of the invention may also cause neurotoxicity at certain doses, the methods herein can be used to reduce or avoid such toxicity.

EXAMPLES

The following non-limiting example serves to illustrate the practice of the present invention.

Example 1

IV Dosage Form

[1S-[1R*,3R*(E),7R*,10S*,11R*,12R*,16S*]]-7,11-Dihydroxy-8,8,10,12,16-pentamethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-4-aza-17-oxabicyclo[14.1.0] heptadecane-5,9-dione, 9.86 g, was wetted/partially dissolved with 600 mL of a 9:1 mixture of tertiary butanol and Water for Injection USP which had been pre-cooled to 5 EC. Once the drug powder had become completely wetted, dissolution was completed by the addition of 600 mL of a 1:9 mixture of tertiary butanol and Water for Injection and 766 mL of a 1:1 mixture of tertiary butanol and Water for Injection which likewise had been pre-cooled to 5 EC thereby making the final solution a 1:1 mixture. The dissolution was carried out under protection from light.

The solution formed above was promptly lyophilized in a Virtis INOTOP lyophilizer at −16 EC under light protectant conditions over a period of 48 hours. The resultant lyophilized product (lyophile) was then further dried at 15 EC under high vacuum for 48 hours. No detectable degradation of the drug was observed during these procedures. The lyophile was packaged under sterile conditions into 30 mL vials, each containing 10 mg of drug and standard excess to allow for vial/needle/syringe loss.

The lyophile is reconstituted with 5.5 mL of a 1:1 volume mixture of Dehydrated Alcohol USP and Cremophor EL®, which typically will be supplied with the drug in a separate vial, to achieve a final drug concentration of 2 mg/mL. Once dissolution is effected by gently swirling the vial, the resultant solution is diluted to achieve a concentration of 0.2 mg/mL by the addition of 9 mL of Lactated Ringer's for Injection for each milliliter of constituted drug product.

Example 2

IV Administration of Compound II

A total of 24 cancer patients (12 male and 12 female) received compound II by IV administration to evaluate the maximum tolerated dose (MTD), the dose limiting toxicity (DLT), the pharmacokinitics and pharmacodynamics, and to evaluate the anti-tumor activity of compound II. The median age (range) of the patients was 57 (34–74). 5 patients had breast cancer, 5 patients had head and neck cancer, 2 patients had sarcoma, 2 patients had colorectal cancer, 2 patients had UPT cancer, 2 patients had melanoma, 2 patients had cancer of the esophagus, 1 patient had renal cancer, 1 patient had cervical cancer, 1 patient had thyroid cancer, and 1 patient had anal cancer. 21 patients had received prior chemotherapy (18 patients received neurotoxic agents and 18 patients received radiotherapy). The median number of prior chemotherapy lines including adjuvant (range) was 2 (1–3).

Patients were orally administered compound II on day 1 (for dose levels of 20 mg/m^2 and higher) followed by a 30 minute IV infusion of compound II every week starting on day 7. Patients were administered compound II at doses of 1, 2.5, 5, 10, 20, 25, and 30 mg/m^2. Patients were monitored during the course of treatment for dose limiting toxicity (DLT) The results of the study showed that compound II can be administered weekly at doses up to 30 mg/m^2 without severe toxicity being observed.

In a second study a total of 12 cancer patients (5 male and 7 female) were orally administered compound II on day 1 followed by a 30 minute IV infusion of compound II every week starting on day 7 at a dose of 25 mg/m^2 to evaluate neurotoxicity of compound II. The median age (range) of the patients was 51 (30–65). 4 patients had colorectal cancer, 3 patients had breast cancer, 2 patients had melanoma, 1 patient had renal cancer, 1 patient had sarcoma, and 1 patient had ovary cancer. 10 patients had received prior chemotherapy (6 patients received neurotoxic agents and 18 patients received radiotherapy). The median number of prior chemotherapy lines including adjuvant (range) was 2 (0–3). This study showed that compound II can be used to patients

that have had prior chemotherapy lines that include the use of neurotoxic anticancer agents. In patients that have had prior chemotherapy lines that use neurotoxic anticancer agents, however, it is preferably, that the cumulative dose of compound II does not exceed about 200 mg/m^2 per cycle.

The study further showed that breast tumors and colon tumors, in patients previously treated with chemotherapy, responded to treatment with compound II. Specifically, breast cancer patients previously treated with adriamycin and taxotere with cyclophosphamide, 5-fluorouracil, methotrexate adjuvant therapy; adriamycin and taxotere with cyclophosphamide, 5-fluorouracil, methotrexate adjuvant therapy; or adriamycin, cyclophosphamide, 5-fluorouracil for metastatic cancer responded to treatment with compound II. Patients with metastatic colon cancer previously treated with taxol and carboplatin; 5-fluorouracil and leucovorin; or irinotecan responded to treatment with compound II.

Example 3

Pharmacokinetics of Compound II Orally Administered to Cancer Patients

Patients with Advanced Malignancies were administered Compound II weekly as a 30-minute infusion (a course=3 intravenous weekly administrations). Patients received doses of 1, 2.5, 5, 10, 20, 25, or 30 mg/m^2. Starting at the 20 mg/m^2 dose level, a single oral dose of Compound II was given on day 6 in a vehicle of 80% propylene glycol and 20% ethanol (v/v) followed by administration of a citrate/phosphate buffer (22.5 gm) before course 1 to assess the absolute bioavailability of Compound II. The dose of oral Compound II administered on Day 6 matched the dose of IV Compound II administered on day 1. Serial plasma sampling was obtained on day 6 and day 1 of course 1 to assess pharmacokinetics by an LC/MS/MS.

Samples were analyzed by adding an internal standard to 0.2 mL of plasma sample, precipitating with acetone, and then extracting the supernatant with 1-chlorobutane. The organic layer was removed and evaporated to dryness. The residue was reconstituted and injected into the LC/MS/MS system. Chromatographic separation was achieved, isocratically, on a YMC ODS-AQ column (4.6×50 mm, 3:m) with a mobile phase of acetonitrile:0.01M ammonium acetate, pH 5.0 (65:35). Detection was by negative electrospray tandem mass spectrometry. The standard curve, which ranged from 2 to 500 ng/mL for all analytes and was fitted to a 1/x weighted quadratic regression model.

Compound II for oral administration, 25 mg/vial, was supplied as "drug in bottle." The vehicle (buffer) for constitution of Compound II, 25 mg/vial, was a mixture of 80% propylene glycol and 20% ethanol (v/v). The propylene glycol/ethanol mixture was prepared by mixing 80 parts by volume of propylene glycol and 20 parts by volume of ethanol in a suitable container and gently swirling the container until the solution was completely mixed.

The citrate/phosphate buffer for oral administration after compound II was supplied in a separate bottle. Buffer for use with Compound II was constituted with water for injection (WFI).

Compound II was prepared for administration to patients by using a suitable syringe to slowly inject 2.5, 5, or 10 mL of the propylene glycol/ethanol mixture into the 20 cc vial containing 25 mg/vial of Compound II, to give concentrations of 10, 5, or 2.5 mg/mL, respectively, depending on the dose to be administered to the patient. The syringe was removed and the vial shaken vigorously for 10 seconds. The vial was placed in a sonication bath and sonicated until the solution became clear. Vials were pooled depending on the dose.

The buffer for administration with Compound II was supplied in an 8 oz. clear glass bottle and was constituted with water for injection (WFI). The child resistant cap was removed from the bottle of buffer and about 140 mL of water for injection (WFI) were added. The bottle was shaken vigorously or sonicated with intermittent shaking until a clear solution was obtained.

Following oral administration on day 6, 7 mL blood samples was collected into Becton Dickinson Vacutainer tubes with K3EDTA as anticoagulent (lavender-colored top) according to the following schedule (expressed as hours:minutes from the start of the oral administration): predose, 00:15, 00:30, 00:45, 1:00, 1:30, 2:00, 3:00, 4:00, 6:00, 8:00, 24:00, 48:00, and 72:00. Following IV administration on day 1, 7 mL blood samples were collected into Becton Dickinson Vacutainer tubes with K3EDTA as anticoagulent (lavender-colored top) according to the following schedule (expressed as hours:minutes from the start of the IV infusion):predose, 00:15, 00:30 (end-of infusion), 00:45, 1:00, 1:30, 2:00, 3:00, 4:00, 6:00, 8:00, 24:00, 48:00, and 72:00.

Immediately after blood collection, the Vacutainer tubes were inverted several times to ensure mixing with the anticoagulant and then immediately placed on crushed ice. Within 30 minutes of collection, samples were centrifuged for 5 minutes at approximately 2000×g and 0 to 5 EC. The plasma was then transferred to separate pre-labeled screw-capped polypropylene tubes and stored at −70 EC until bioanalysis. Plasma concentrations of Compound II were analyzed using the LC/MS/MS assay.

The plasma concentration versus time data were analyzed using non-compartmental methods. The pharmacokinetic parameters determined for Compound II included the maximum observed plasma concentration (Cmax), time to reach Cmax (Tmax), area under the plasma concentration time curve from time zero to the time of last sampling time T(AUC(0-T)).

A total of 18 patients have received oral Compound II as a solution on day 6 and by IV on day 1. The summary of the pharmacokinetic results from these patients is presented in Table 1.

TABLE 1

Summary of Pharmacokinetics of Patients Administered Compound II Orally and Intravenously

Dose (mg/m^2)	20		25		30	
N	3		11		4	
Route	IV	Oral	IV	Oral	IV	Oral
Formulation	IV	Solution for Oral Admin.	IV	Solution for Oral Admin.	IV	Solution for Oral Admin.
CMAX[a] (ng/mL)	251 (108)	142 (106)	447 (189)	180 (110)	711 (530)	274 (104)
TMAX[b] (h)	0.25 (0.25, 0.25)	1.0 (0.25, 1.50)	0.50 (0.25, 0.50)	0.50 (0.25, 3.00)	0.50 (0.25, 0.50)	0.50 (0.25, 0.75)
AUC(0-T)[a,c] (H · ng/mL)	796 (587)	404 (381)	848 (284)	533 (577)	1155 (292)	708 (291)
% F[a]	NA	43.5 (16.1)	NA	55.6 (18.4)	NA	62.2 (25.1)

[a]Mean (SD)
[b]Median (min, max)
[c]Represents AUC(0-T)

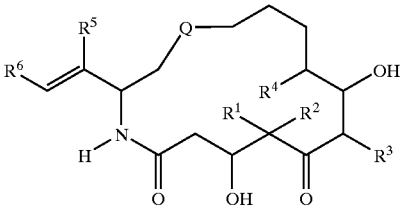
The embodiments of the invention described above are intended to be merely exemplary, and those skilled in the art will recognize, or will be able to ascertain using no more

than routine experimentation, numerous equivalents of specific compounds, materials, and procedures. All such equivalents are considered to be within the scope of the invention and are encompassed by the appended claims.

What is claimed is:

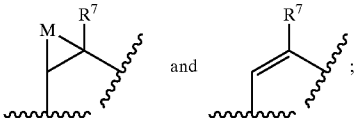
1. A process for formulating, for parenteral administration, an epothilone analog represented by formula I:

I



wherein:

Q is selected from the group consisting of:



and

M is selected from the group consisting of oxygen, sulfur, NR^8, and CR^9R^{10};

each R^1, R^2, R^3, R^4, R^5, R^7, R^{11}, R^{12}, R^{13}, R^{14}, and R^{15} is, independently, selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, substituted aryl and heterocyclo, and wherein R^1, and R^2, are alkyl, they can be joined to form cycloalkyl;

R^6, is selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, cycloalkyl, heterocyclo and substituted heterocyclo;

R^8 is selected from the group consisting of hydrogen, alkyl, substituted alkyl, R^{14}, C═O, $R^{12}OC$═O and $R^{13}SO_2$; and

each R^9 and R^{10} is, independently, selected from the group consisting of hydrogen, halogen, alkyl, substituted alkyl, aryl, heterocyclo, hydroxy, $R^{14}C$═O, and $R^{15}OC$═O;

and any salts, solvates, or hydrates thereof, comprising the following steps carried out under protection from light:

a) dissolving said epothilone analog in a mixture of at least about 50% by volume tertiary-butanol in water to form a solution;

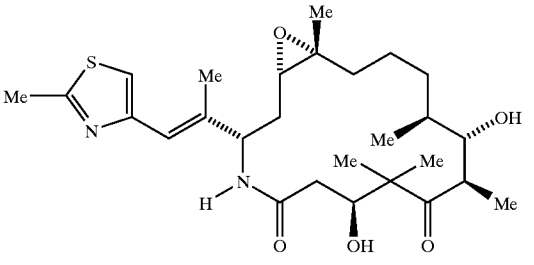
b) performing primary drying of said solution at a temperature of from about −10° C. to about −40° C. under high vacuum of from about 50 millitorr to about 300 millitorr for from about 24 hours to about 96 hours to form a dried product;

c) performing secondary drying of the resultant dried product at a temperature of from about 10° C. to about 30° C. under high vacuum of from about 50 millitorr to about 300 millitorr for from 24 hours to about 96 hours to provide a lyophilized product; and

d) packaging said lyophilized product in a first vial in combination with a second vial containing a sufficient quantity of an equal mixture by volume of a suitable nonionic surfactant and anhydrous ethanol to effect solution thereof.

2. The process of claim **1** wherein said epothilone analog is represented by formula II:

II.



3. The process of claim **1** wherein in step a) said analog is first wetted with a mixture of at least about 60% tertiary-butanol in water, and then sufficient water, or a mixture of tertiary-butanol and water, is added thereto so that the resulting solution contains from about 2 mg/mL to about 30 mg/mL of said analog in a mixture of from about 50% to about 80% by volume tertiary-butanol in water.

4. The process of claim **2** wherein in step a) said analog is first wetted with a mixture of at least about 60% tertiary-butanol in water, and then sufficient water, or a mixture of tertiary-butanol and water, is added thereto so that the resulting solution contains from about 2 mg/mL to about 30 mg/mL of said analog in a mixture of from about 50% to about 80% by volume tertiary-butanol in water.

5. The process of claim **3** wherein in step a) said analog is initially wetted with a mixture of from about 60% to about 95% by volume tertiary-butanol in water.

6. The process of claim **4** wherein in step a) said analog is initially wetted with a mixture of from about 60% to about 95% by volume tertiary-butanol in water.

7. The process of claim **1** wherein said primary drying in step b) is carried out at a temperature of about −25° C. and a pressure of about 200 millitorr for about 48 hours.

8. The process of claim **2** wherein said primary drying in step b) is carried out at a temperature of about −25° C. and a pressure of about 200 millitorr for about 48 hours.

9. The process of claim **1** wherein said secondary drying in step c) is carried out at a temperature of about 25° C. and a pressure of about 150 millitorr for about 48 hours.

10. The process of claim **2** wherein said secondary drying in step c) is carried out at a temperature of about 25° C. and a pressure of about 150 millitorr for about 48 hours.

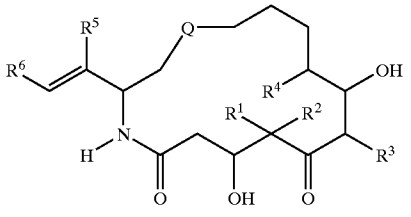
11. The process of claim **1** wherein said surfactant is polyethoxylated castor oil.

12. The process of claim **2** wherein said surfactant is polyethoxylated castor oil.

13. The process of claim **11** wherein said second vial contains an amount of said mixture sufficient to form a solution of from about 2 mg/mL to about 4 mg/mL of said analog therein.

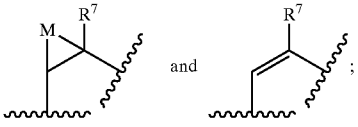
14. The process of claim **12** wherein said second vial contains an amount of said mixture sufficient to form a solution of from about 2 mg/mL to about 4 mg/mL of said analog therein.

15. A pharmaceutical preparation comprising, a first vial containing a lyophilized epothilone analog and a second vial containing a quantity of a solvent for the lyophilized epothilone said solvent comprising a mixture of about equal parts by volume of dehydrated ethanol and a suitable nonionic surfactant, said analog being represented by formula I:



wherein:

Q is selected from the group consisting of



M is selected from the group consisting of oxygen, sulfur, NR^8, and CR^9R^{10};

each R^1, R^2, R^3, R^4, R^5, R^7, R^{11}, R^{12}, R^{13}, R^{14}, and R^{15} is, independently, selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, substituted aryl and heterocyclo, and wherein R^1 and R^2 are alkyl, they can be joined to form cycloalkyl;

R^6 is selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, cycloalkyl, heterocyclo and substituted heterocyclo;

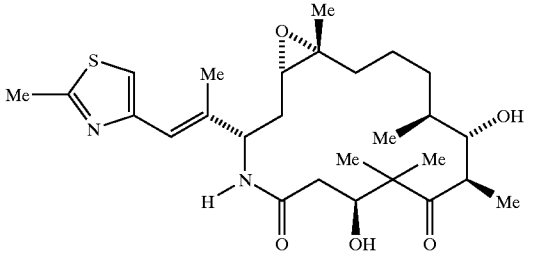
R^8 is selected from the group consisting of hydrogen, alkyl, substituted alkyl, $R^{11}C{=}O$, $R^{12}OC{=}O$ and $R^{13}SO_2$; and

each R^9 and R^{10} is, independently, selected from the group consisting of hydrogen, halogen, alkyl, substituted alkyl, aryl, heterocyclo, hydroxy, $R^{14}C{=}O$, and $R^{15}OC{=}O$;

and any salts, solvates, or hydrates thereof.

16. The pharmaceutical preparation of claim **15** wherein said epothilone analog is represented by formula II:

II.



17. The pharmaceutical preparation of claim **15** wherein said nonionic surfactant is polyethoxylated castor oil.

18. A process for forming a pharmaceutical composition for parenteral administration comprising mixing the contents of the vials of the pharmaceutical preparation of claim **15** to effect solution of said lyophilized epothilone analog and diluting the resultant solution with a quantity of a suitable parenteral diluent such that the concentration of said analog therein will be from about 0.1 mg/mL to about 0.9 mg/mL.

19. A process for forming a pharmaceutical composition for parenteral administration comprising mixing the contents of the vials of the pharmaceutical preparation of claim **16** to effect solution of said lyophilized epothilone analog and diluting the resultant solution with a quantity of a suitable parenteral diluent such that the concentration of said analog therein will be from about 0.1 mg/mL to about 0.9 mg/mL.

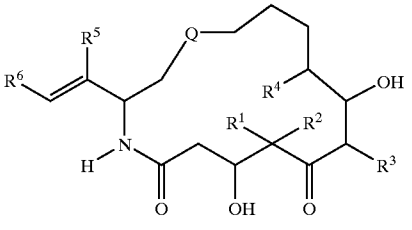
20. A process for forming a pharmaceutical composition for parenteral administration comprising mixing the contents of the vials of the pharmaceutical preparation of claim **17** to effect solution of said lyophilized epothilone analog and diluting the resultant solution with a quantity of a suitable parenteral diluent such that the concentration of said analog therein will be from about 0.1 mg/mL to about 0.9 mg/mL.

21. The process of claim **18** wherein said diluent is Lactated Ringer's Injection.

22. The process of claim **19** wherein said diluent is Lactated Ringer's Injection.

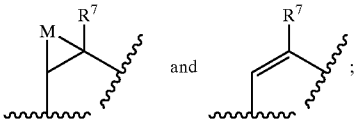
23. The process of claim **20** wherein said diluent is Lactated Ringer's Injection.

24. A method for treating a patient in need of treatment with an epothilone analog represented formula I:



wherein:

Q is selected from the group consisting of M,



M is selected from the group consisting of oxygen, sulfur, NR^8, and CR^9R^{10};

each R^1, R^2, R^3, R^4, R^5, R^7, R^{11}, R^{12}, R^{13}, R^{14}, and R^{15} is, independently, selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, substituted aryl and heterocyclo, and wherein R^1 and R^2 are alkyl, they can be joined to form a cycloalkyl;

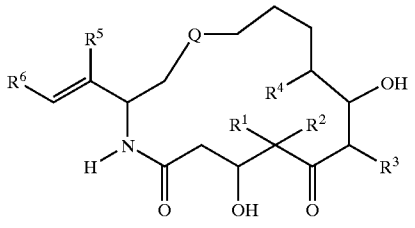
R^6 is selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, cycloalkyl, heterocyclo and substituted heterocyclo;

R^8 is selected from the group consisting of hydrogen, alkyl, substituted alkyl, $R^{11}C{=}O$, $R^{12}OC{=}O$ and $R^{13}SO_2$, and

each R^9 and R^{10} is, independently, selected from the group consisting of hydrogen, halogen, alkyl, substituted alkyl, aryl, heterocyclo, hydroxy, $R^{14}C{=}O$, and $R^{15}OC{=}O$;

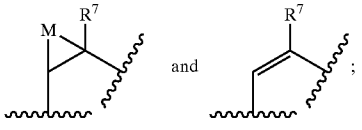
and any salts, solvates, or hydrates thereof, comprising administering to said patient, by intravenous injection or infusion, an effective amount of a pharmaceutical composition of claim **18**.

25. A method for treating a patient in need of treatment with an epothilone analog represented formula I:



wherein:

Q is selected from the group consisting of



M is selected from the group consisting of oxygen, sulfur, NR^8 and CR^9R^{10};

each R^1, R^2, R^3, R^4, R^5, R^7, R^{11}, R^{13}, R^{14}, and R^{15} is, independently, selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, substituted aryl and heterocyclo, and wherein R' and R^2 are alkyl, they can be joined to form a cycloalkyl; and

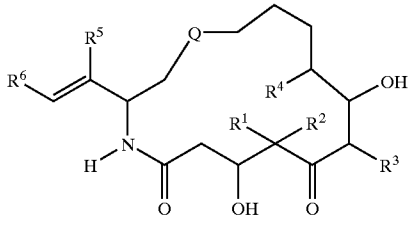
R^6 is selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, cycloalkyl, heterocyclo and substituted heterocyclo;

R^8 is selected from the group consisting of hydrogen, alkyl, substituted alkyl, R^{11}, C═O, $R^{12}OC$═O and $R^{13}SO_2$; and

each R^9 and R^{10} is, independently, selected from the group consisting of hydrogen, halogen, alkyl, substituted alkyl, aryl, heterocyclo, hydroxy, $R^{14}C$═O, and $R^{15}OC$═O;

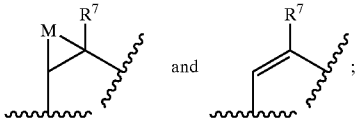
and any salts, solvates, or hydrates thereof, comprising administering to said patient, by intravenous injection or infusion, an effective amount of a pharmaceutical composition of claim **19**.

26. A method for treating a patient in need of treatment with an epothilone analog represented formula I:



wherein:

Q is selected from the group consisting of



M is selected from the group consisting of oxygen, sulfur, NR^8 and CR^9R^{10};

each R', R^2, R^3, R^4, R^5, R^7, R^{11}, R^{12}, R^{13}, R^{14}, and R^{15} is, independently, selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, substituted aryl and heterocyclo, and wherein R^1 and R^2 are alkyl, they can be joined to form a cycloalkyl;

R^6 is selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, cycloalkyl, heterocyclo and substituted heterocyclo; R^8 is selected from the group consisting of hydrogen, alkyl, substituted alkyl, $R^{11}C$═O, $R^{12}OC$═O and $R^{13}SO_2$; and

each R^9 and R^{10} is, independently, selected from the group consisting of hydrogen, halogen, alkyl, substituted alkyl, aryl, heterocyclo, hydroxy, $R^{14C—O,}$ and $R^{15}OC$═O;

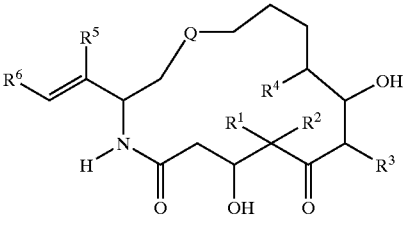
and any salts, solvates, or hydrates thereof, comprising administering to said patient, by intravenous injection or infusion, an effective amount of a pharmaceutical composition of claim **20**.

27. The method of claim **24** wherein said diluent is Lactated Ringer's Injection.

28. The method of claim **25** wherein said diluent is Lactated Ringer's Injection.

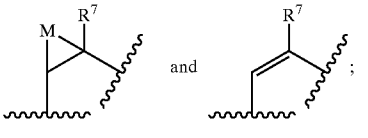
29. The method of claim **26** wherein said diluent is Lactated Ringer's Injection.

30. A pharmaceutical composition suitable for parenteral administration comprising in lyophilized form a compound represented by formula I:



wherein:

Q is selected from the group consisting of



M is selected from the group consisting of oxygen, sulfur, NR^8, and CR^9R^{10};

each R', R^2, R^3, R^4, R^5, R^7, R^{11}, R^{12}, R^{13}, R^{14} and R^{15} independently, selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, substituted aryl and heterocyclo, and wherein R' and R^2 are alkyl, they can be joined to form a cycloalkyl;

R^6 is selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, cycloalkyl, heterocyclo and substituted heterocyclo;

R^8 is selected from the group consisting of hydrogen, alkyl, substituted alkyl, $R^{11}C$═O, R^2OC═O and $R^{13}SO_2$; and

each R^9 and R^{10} is, independently, selected from the group consisting of hydrogen, halogen, alkyl, substituted alkyl, aryl, heterocyclo, hydroxy, $R^{14}C$═O, and $R^{15}OC$═O;

and any salts, solvates, or hydrates thereof;
dehydrated alcohol;
and a non-ionic surfactant.

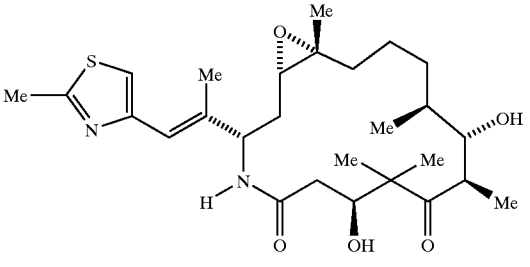
31. The composition of claim **30**,wherein the surfactant is polyethoxylated castor oil.

32. The composition of claim **30**, wherein the surfactant is Cremophor EL®.

33. The composition of claim **30**, wherein the concentration of the compound of formula I is from about 2 mg/mL to 4 mg/mL.

34. A pharmaceutical composition suitable for parenteral administration comprising a compound represented by formula II:

II



and any salts, solvates, or hydrates thereof:
dehydrated alcohol; and
a non-ionic surfactant.

35. A method of treating cancer in a patient comprising intravenously administering to said patient a therapeutically effective amount of the pharmaceutical formulation of claim **30** diluted in a parenteral diluent.

36. The method of claim **35**, wherein the parenteral diluent is 5% dextrose, lactated ringer's and dextrose injection, or sterile water for injection.

37. The method of claim **35**, wherein the concentration of the compound of formula I in the parenteral diluent is about 0.1 mg/mL to 0.9 mg/mL.

38. The method of claim **35**, wherein the compound of formula I is administered in a dose of about 1 mg/m^2 to 65 mg/m^2.

39. The method of claim **35**, wherein the compound of formula I is administered at a dose of about 25 mg/m^2.

40. The method of claim **35**, wherein the pharmaceutical composition is administered weekly as an IV infusion.

41. The method of claim **35**, wherein the IV infusion is administered over a period of about 45 minutes to 90 minutes.

42. The method of claim **35**, wherein the IV infusion is administered over a period of about 1 hour.

43. The method of claim **35**, further comprising administering to said patient one or more additional agents to prevent nausea, vomiting, hypersensitivity, or gastric irritation.

44. The method of claim **43**, wherein the one or more additional agents is an H1 or H2 antihistamine.

45. The method of claim **35**, wherein the patient has not previously been treated for cancer.

46. The method of claim **35**, wherein the patient has been previously treated for cancer.

47. The method of claim **35**, wherein the cancer is refractory to radiation therapy.

48. The method of claim **35**, wherein the cancer is refractory to anti-cancer chemotherapy.

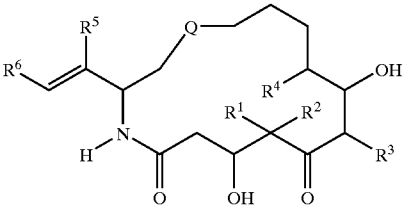
49. A method of treating cancer in a patient previously experiencing neurotoxicity comprising intravenously administering to said patient a therapeutically effective amount of the pharmaceutical formulation of claim **30** diluted in a parenteral diluent as a weekly infusion, wherein the total dose of the compound of formula I is less than about 200 mg/m^2.

50. The method of claim **35**, wherein the cancer is a solid tumor.

51. The method of claim **29**, wherein the cancer is a solid tumor.

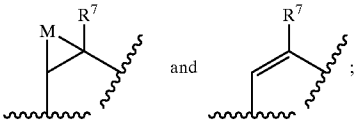
52. A method of treating cancer while reducing or avoiding neurotoxicity which comprises intravenously administering a therapeutically effective amount of compound represented by formula I:

I



wherein:

Q is selected from the group consisting of



M is selected from the group consisting of oxygen, sulfur, NR^8 and CR^9, R^{10},

each R^1, R^2, R^3, R^4, R^5, R^7, R^{11}, R^{12}, R^{13}, R^{14} and R^{15} is, independently, selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, substituted aryl and heterocyclo, and wherein R' and R^2 are alkyl, they can be joined to form a cycloalkyl;

R^6 is selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, cycloalkyl, heterocyclo and substituted heterocyclo;

R^8 is selected from the group consisting of hydrogen, alkyl, substituted alkyl, $R^{11}C{=}O$, $R^{12}OC{=}O$ and $R^{12}SO_2$ and

each R^9 and R^{10} is, independently, selected from the group consisting of hydrogen, halogen, alkyl, substituted alkyl, aryl, heterocyclo, hydroxy, $R^{14}C{=}O$, and $R^{15}OC{=}O$;

and any salts, solvates, or hydrates thereof;

over a period of one (1) hour to a patient in need thereof.

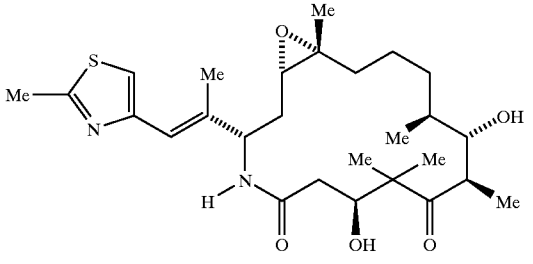
53. The method of claim **52**, wherein the infusion is made on a weekly basis.

54. The method of claim **52**, wherein the therapeutically effective amount is from about 1 mg/m^2 to about 65 mg/m^2.

55. The method of claim **54**, wherein the amount is 25 mg/m^2.

56. A method of treating cancer while reducing or avoiding neurotoxicity which comprises intravenously infusing a therapeutically effective amount of compound represented by formula I:

II



over a period of one (1) hour to a patient in need thereof.

57. The method of claim **56** which further comprises orally administering said compound 1 week before or after an intravenous administration.

58. A method of treating cancer in a human patient in need thereof with a synthetic or semi-synthetic epothilone analogue that is active against cancer which comprises a four (4) week dosing cycle wherein said cycle comprises three weeks of weekly intravenous administration and one week of oral administration of said epothilone analogue.

59. The method of claim **58** wherein the compound is administered daily for 3 days with a period of 1 week to 3 weeks between cycles where there is no administration of the compound.

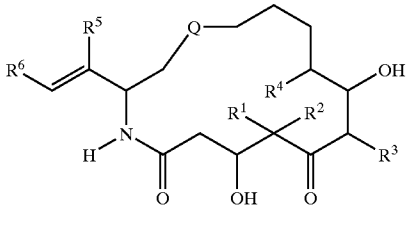
60. The method of claim **58** wherein the compound is administered daily for 3 days with a period of 1 week to 3 weeks between cycles where there is no administration of the compound.

61. The method of claim **58** wherein the compound is administered daily for 5 days with a period of 1 week to 3 weeks between cycles where there is no administration of the compound.

62. The method of claim **58** wherein the compound is administered daily for 5 days with a period of 1 week to 3 weeks between cycles where there is no administration of the compound.

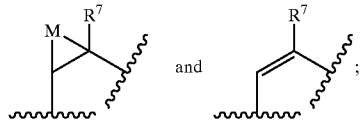
63. A method of treating cancer in a patient comprising orally administering to said patient daily for 3 days, daily for 5 days, or weekly a therapeutically effective amount of a compound represented by formula I:

I



wherein:

Q is selected from the group consisting of



M is selected from the group consisting of oxygen, sulfur, NR^8 and CR^9R^{10};

each R^1, R^2, R^3, R^4, R^5, R^7 R^{11} R^{12} R^{13} R^{14} and R^{15} is, independently, selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, substituted aryl and heterocyclo, and wherein R^1 and R^2 are alkyl, they can be joined to form a cycloalkyl;

R^6 is selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, cycloalkyl, heterocyclo and substituted heterocyclo;

R^8 is selected from the group consisting of hydrogen, alkyl, substituted alkyl, $R^{11}C{=}O$, $R^{12}OC{=}O$ and $R^{13}SO_2$; and

each R^9 and R^{10} is, independently, selected from the group consisting of hydrogen, halogen, alkyl, substituted alkyl, aryl, heterocyclo, hydroxy, $R^{14}C{=}O$, and $R^{15}OC{=}O$;

and any salts, solvates, or hydrates thereof.

64. The method of claim **63**, wherein the compound of formula I is administered in a dose of about 0.05 mg/kg to 200 mg/kg.

65. The method of claim **64**, wherein the compound of formula I is administered at a dose of about 1 mg/m^2 to 65 mg/m^2.

66. The method of claim **64**, wherein the compound is administered every 3 weeks.

67. The method of claim **63** wherein the compound is administered daily for 3 days with a period of 1 week to 3 weeks between cycles where there is no administration of the compound.

68. The method of clam **66** wherein the compound is administered daily for 5 days with a period of 1 week to 3 weeks between cycles where there is no administration of the compound.

69. The method of claim **66** wherein the compound is administered daily for 3 days with a period of 4 days between cycles where there is no treatment.

70. The method of claim **66** wherein the compound is administered daily for 5 days with a period of 2 days between cycles where there is no treatment.

71. The pharmaceutical preparation of claim **1**, wherein the lyophilized epothilone analog is free of excipients.

72. The pharmaceutical preparation of claim **15**, wherein the lyophilized epothilone analog is free of excipients.

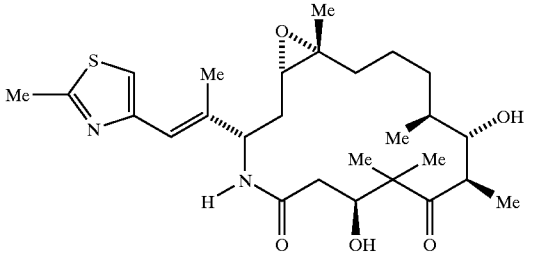
73. The pharmaceutical preparation of claim **16**, wherein the lyophilized epothilone analog is free of excipients.

74. A process for forming a pharmaceutical composition for parenteral administration comprising mixing the contents of the vials of the pharmaceutical preparation of claim **72** to effect solution of said lyophilized epothilone analog and diluting the resultant solution with a quantity of a suitable parenteral diluent such that the concentration of said analog therein will be from about 0.1 mg/mL to about 0.9 mg/mL.

75. A process for forming a pharmaceutical composition for parenteral administration comprising mixing the contents of the vials of the pharmaceutical preparation of claim **73** to effect solution of said lyophilized epothilone analog and diluting the resultant solution with a quantity of a suitable parenteral diluent such that the concentration of said analog therein will be from about 0.1 mg/mL to about 0.9 mg/mL.

76. A method of treating cancer in a patient comprising intravenously and orally administering to said patient a therapeutically effective amount of a compound represented by formula II:

II.

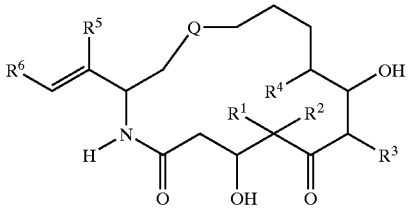


77. A method of treating cancer in a patient comprising intravenously administering to said patient a therapeutically effective amount of the compound of claim **76** diluted in a parenteral diluent.

78. The pharmaceutical preparation of claim **15**, wherein the quantity of solvent is an amount such that when the solvent is combined with the lyophilized epothilone the resulting solution contains from about 2 mg/mL to about 4 mg/mL of said analog.

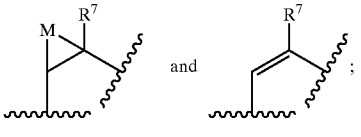
79. A method of treating cancer in a patient comprising intravenously administering to said patient daily for 3 days or daily for 5 days a therapeutically effective amount of a compound represented by formula I:

I



wherein:

Q is selected from the group consisting of



M is selected from the group consisting of oxygen, sulfur, NR, and CR^9R^{10};

each R^1, R^2, R^3, R^4, R^5, R^7, R^{11}, R^{12}, R^{13}, R^{14}, and R^{15}, is, independently, selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, substituted aryl and heterocyclo, and wherein R^1 and R^2 are alkyl, they can be joined to form a cycloalkyl;

R^6 is selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, cycloalkyl, heterocyclo and substituted heterocyclo;

R^8 is selected from the group consisting of hydrogen, alkyl, substituted alkyl, $R^{11}C{=}O$, $R^{12}OC{=}O$ and $R^{13}SO_2$; and

each R^9 and R^{10} is, independently, selected from the group consisting of hydrogen, halogen, alkyl, substituted alkyl, aryl, heterocyclo, hydroxy, $R^{14}C{=}O$, and $R^{15}OC{=}O$;

and any salts, solvates, or hydrates thereof.

80. The method of claim **79**, wherein the compound of formula I is administered daily for 3 days.

81. The method of claim **79**, wherein the compound of formula I is administered daily for 5 days.

82. The method of claim **81**, wherein the compound of formula I is administered in a dose of about 0.05 mg/kg to 200 mg/kg.

83. The method of claim **79**, wherein the compound of formula I is administered at a dose of about 1 mg/m^2 to 65 mg/m^2.

84. The method of claim **83**, wherein the compound of formula I is administered at a dose of about 25 mg/m^2.

85. The method of claim **79**, wherein the IV infusion is administered over a period of about 45 minutes to 90 minutes.

86. The method of claim **79**, wherein the IV infusion is administered over a perioe of about 1 hour.

87. The method of claim **79**, further comprising administering to said patient one or more additional therapeutic agents to prevent nausea, vomiting, hypersensitivity, or gastric irritation.

88. The method of claim **86**, wherein the one or more additional therapeutic agents is an H^1, or H^2, antihistamine.

89. The method of claim **79**, wherein the patient has not previously been treated for cancer.

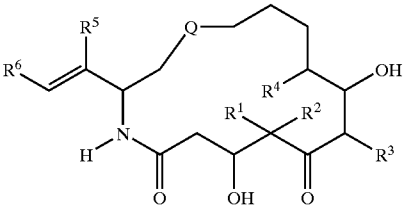
90. The method of claim **86**, wherein the patient has been previously treated for cancer.

91. The method of claim **79**, wherein the cancer is refractory to radiation therapy.

92. The method of **79**, wherein the cancer is refractory to anti-cancer chemotherapy.

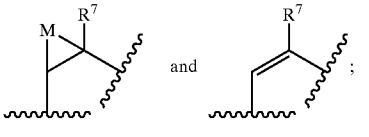
93. A method of treating cancer in a patient comprising intravenously administering to said patient every week or every 3 weeks a therapeutically effective amount of a compound represented by formula I:

I



wherein:

Q is selected from the group consisting of



M is selected from the group consisting of oxygen, sulfur, NR, and CR^9R^{10};

each R^1, R^2, R^3, R^4, R^5, R^7, R^{11}, R^{12}, R^{13}, R^{14}, and R^{15}, is, independently, selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, substituted aryl and heterocyclo, and wherein R^1 and R^2 are alkyl, they can be joined to form a cycloalkyl;

R^6 is selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, cycloalkyl, heterocyclo and substituted heterocyclo;

R^8 is selected from the group consisting of hydrogen, alkyl, substituted alkyl, $R^{11}C{=}O$, $R^{12}OC{=}O$ and $R^{13}SO_2$; and

each R^9 and R^{10} is, independently, selected from the group consisting of hydrogen, halogen, alkyl, substituted alkyl, aryl, heterocyclo, hydroxy, $R^{14}C{=}O$, and $R^{15}OC{=}O$;

and any salts, solvates, or hydrates thereof.

94. The method of **93**, wherein the compound of formula I is administered every week.

95. The method of **93**, wherein the compound of formula I is administered every 3 weeks.

96. The method of claim **95**, further comprising orally administering the compound of formula I before the 3 week cycle.

97. The method of claim **95**, further comprising orally administering the compound of formula I after the 3 week cycle.

98. The method of claim **97**, wherein the compound of formula I is administered as one or more 28 day cycles, wherein the compound of formula I is administered as an IV infusion on days 1, 7, and 14 and orally on day 21.

99. The method of claim **93**, wherein the IV infusion is administered over a period of about 1 hour.

100. The method of claim **93**, further comprising administering to said patient one or more additional therapeutic agents to prevent nausea, vomiting, hypersensitivity, or gastric irritation.

101. The method of claim **100**, wherein the one or more additional therapeutic agents is an H^1, or H^2, antihistamine.

102. The method of claim **93**, wherein the patient has not previously been treated for cancer.

103. The method of claim **93**, wherein the patient has been previously treated for cancer.

104. The method of claim **93**, wherein the cancer is refractory to radiation therapy.

105. The method of claim **93**, wherein the cancer is refractory to anti-cancer chemotherapy.

* * * * *

Disclaimer

6,670,384 B2 — Rebanta Bandyopadhyay, Portage, MI (US); Timothy M. Malloy, Yardley, PA (US); Andrea Panaggio, West Windsor, NJ (US); Krishnaswamy Srinivas Raghavan, Cranbury, NJ (US); Sailesh Amilal Varia, Princeton Junction, NJ (US). Patent dated Dec. 30, 2003. Disclaimer filed May 16, 2005, by the assignee, Bristol-Myers Squibb Company.

The term of this patent shall not extend beyond the expiration date of Patent No. 6,576,651.

(Official Gazette, April 22, 2008)

US006670384C1

(12) EX PARTE REEXAMINATION CERTIFICATE (5663rd)

United States Patent
Bandyopadhyay et al.

(10) **Number: US 6,670,384 C1**
(45) **Certificate Issued: *Jan. 23, 2007**

(54) **METHODS OF ADMINISTERING EPOTHILONE ANALOGS FOR THE TREATMENT OF CANCER**

(75) Inventors: **Rebanta Bandyopadhyay**, Portage, MI (US); **Timothy M. Malloy**, Yardley, PA (US); **Andrea Panaggio**, West Windsor, NJ (US); **Krishnaswamy Srinivas Raghavan**, Cranbury, NJ (US); **Sailesh Amilal Varia**, Princeton Junction, NJ (US)

(73) Assignee: **Bristol-Myers Squibb Company**, Princeton, NJ (US)

Reexamination Request:
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Issued: **Dec. 30, 2003**
Appl. No.: **10/055,653**
Filed: **Jan. 23, 2002**

(*) Notice: This patent is subject to a terminal disclaimer.

Related U.S. Application Data

(60) Provisional application No. 60/264,228, filed on Jan. 25, 2001, and provisional application No. 60/290,008, filed on May 11, 2001.

(51) **Int. Cl.**
A61K 31/365 (2006.01)

(52) **U.S. Cl.** **514/365**; 514/183

(58) **Field of Classification Search** ..... None
See application file for complete search history.

(56) **References Cited**

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Primary Examiner—Phyllis G. Spivack

(57) **ABSTRACT**

A process for formulating certain epothilone analogs for parenteral administration is disclosed wherein the analog is dissolved in a mixture of at least 50% by volume tertiary-butanol in water, the mixture is lyophilized, the resulting lyophilized product is packaged in one vial with a sufficient amount of solvent comprising anhydrous ethanol and a suitable nonionic surfactant in a second vial. All steps are carried out with protection from light. In use, the contents of the second or diluent vial are added to the lyophilized product and mixed to constitute the epothilone analog and the resulting solution is diluted with a suitable diluent to produce a solution for intravenous injection containing the epothilone analog in a concentration of from about 0.1 mg/mL to about 0.9 mg/mL. A preferred surfactant is polyethoxylated castor oil and a preferred diluent is Lactated Ringer's Injection.

EX PARTE REEXAMINATION CERTIFICATE ISSUED UNDER 35 U.S.C. 307

THE PATENT IS HEREBY AMENDED AS INDICATED BELOW.

Matter enclosed in heavy brackets [ ] appeared in the patent, but has been deleted and is no longer a part of the patent; matter printed in italics indicates additions made to the patent.

ONLY THOSE PARAGRAPHS OF THE SPECIFICATION AFFECTED BY AMENDMENT ARE PRINTED HEREIN.

Column 2, line 66 to column 3, line 4:

As discussed herein a wide variety of cancers are encompassed by the methods of the present invention. In a preferred embodiment, the methods of the invention are for the treatment of solid tumors including but not limited to breast, head and neck, sarcoma, colorectal, UPT, melanoma, [oesophagus] *esophagus*, renal, cervix, thyroid, anal, ovarian, and colon.

Column 3, lines 57–61:

each R^1, R^2, R^3, R^4, R^5, R^7, R^{11}, R^{12}, R^{13} [and] R^{14} *and* R^{15} is, independently, selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, substituted aryl and heterocyclo, and wherein R^1 and R^2 are alkyl, they can be joined to form cycloalkyl;

Column 4, lines 16–41:

The term "substituted alkyl" refers to an alkyl group substituted by, for example, one to four substituents, such as, halo, trifluoromethyl, trifluoromethoxy, hydroxy, alkoxy, [cycloalkyoxy] *cycloalkoxy*, [heterocylooxy] *heterocyclyloxy*, oxo, alkanoyl, aryl, aryloxy, aralkyl, alkanoyloxy, amino, alkylamino, arylamino, aralkylamino, cycloalkylamino, [heterocycloamino] *heterocyclylamino*, disubstituted amino in which the two substituents on the amino group are selected from alkyl, aryl, aralkyl, alkanoylamino, aroylamino, aralkanoylamino, substituted alkanoylamino, substituted arylamino, substituted aralkanoylamino, thiol, alkylthio, arylthio, aralkylthio, cycloalkylthio, [heterocyclothio] *heterocyclylthio*, alkylthiono, arylthiono, aralkylthiono, alkylsulfonyl, arylsulfonyl, aralkylsulfonyl, sulfonamido (e.g., SO_2NH_2), substituted sulfonamido, nitro, cyano, carboxy, carbamyl (e.g., $CONH_2$), substituted carbamyl (e.g., CONH alkyl, CONH aryl, CONH aralkyl or instances where there are two substituents on the nitrogen selected from alkyl, aryl or aralkyl), alkoxycarbonyl, aryl, substituted aryl, guanidino and [heterocyclos] *heterocycles*, such as, indolyl, imidazolyl, furyl, thienyl, thiazolyl, [pyrrolidyl] *pyrrolidinyl*, pyridyl, pyrimidyl and the like. Wherein, as noted above, the substituents themselves are further substituted, such further substituents are selected from the group consisting of halogen, alkyl, alkoxy, aryl and aralkyl. The definitions given herein for alkyl and substituted alkyl apply as well to the alkyl portion of alkoxy groups.

Column 4, line 58 to column 5, line 14:

The term "substituted aryl" refers to an aryl group substituted by, for example, one to four substituents such as alkyl; substituted alkyl, halo, trifluoromethyl, trifluoromethoxy, hydroxy, alkoxy, cycloalkyloxy, [heterocyclooxy] *heterocycyloxy*, alkanoyl, alkanoyloxy, amino, alkylamino, dialkylamino, aralkylamino, cycloalkylamino,

[heterocycloamino] *heterocyclylamino*, alkanoylamino, thiol, alkylthio, cycloalkylthio, [heterocyclothio] *heterocyclylthio*, ureido, nitro, cyano, carboxy, carboxyalkyl, carbamyl, alkoxycarbonyl, alkylthiono, arylthiono, alkylsulfonyl, sulfonamido, aryloxy and the like. The substituent may be further substituted by one or more members selected from the group consisting of halo, hydroxy, alkyl, alkoxy, aryl, substituted alkyl, substituted aryl and aralkyl.

Column 5, lines 27–40:

Exemplary monocyclic heterocyclic groups include pyrrolidinyl, pyrrolyl, [indolyl,] pyrazolyl, oxetanyl, pyrazolinyl, imidazolyl, imidazolinyl, imidazolidinyl, oxazolyl, oxazolidinyl, isoxazolinyl, isoxazolyl, thiazolyl, thiadiazolyl, thiazolidinyl, isothiazolyl, isothiazolidinyl, furyl, tetrahydrofuryl, thienyl, oxadiazolyl, piperidinyl, piperazinyl, 2-oxopiperazinyl, 2-oxopiperidinyl, 2-oxopyrrolidinyl, 2-oxazepinyl, azepinyl, 4-piperidonyl, pyridyl, N-oxo-pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, tetrahydropyranyl, tetrahydrothiopyranyl, tetrahydrothiopyranyl sulfone, morpholinyl, thiomorpholinyl, thiomorpholinyl sulfoxide, thiomorpholinyl sulfone, 1,3-dioxolane and tetrahydro-1, 1-dioxothienyl, dioxanyl, isothiazolidinyl, thietanyl, thiiranyl, triazinyl, [and] triazolyl, and the like.

Column 5, lines 41–57:

Exemplary bicyclic heterocyclic groups include *indolyl,* benzothiazolyl, benzoxazolyl, benzothienyl, quinuclidinyl, quinolinyl, quinolinyl-N-oxide, tetrahydroisoquinolinyl, isoquinolinyl, benzimidazolyl, benzopyranyl, indolizinyl, benzofuryl, chromonyl, coumarinyl, cinnolinyl, quinoxalinyl, indazolyl, pyrrolopyridyl, furopyridinyl (such as furo[2,3-c]pyridinyl, [furo[3,1-b]pyridinyl]] *furo[3,1-b] pyridinyl* or furo[2,3-b]pyridinyl), dihydroisoindolyl, dihydroquinazolinyl (such as 3,4-dihydro-4-oxo-quinazolinyl), benzisothiazolyl, benzisoxazolyl, benzodiazinyl, benzofurazanyl, benzothiopyranyl, benzotriazolyl, benzpyrazolyl, dihydrobenzofuryl, dihydrobenzothienyl, dihydrobenzothiopyranyl, dihydrobenzothiopyranyl sulfone, dihydrobenzopyranyl, indolinyl, isochromanyl, isoindolinyl, naphthyridinyl, phthalazinyl, piperonyl, purinyl, pyridopyridyl, quinazolinyl, tetrahydroquinolinyl, thienofuryl, thienopyridyl, thienothienyl, and the like.

Column 6, lines 51–55:

hematopoietic tumors of lymphoid lineage, including leukemia, acute lymphocytic leukemia, acute lymphoblastic leukemia, B-cell lymphoma, T-cell lymphoma, Hodgkins lymphoma, non-Hodgkins lymphoma, hairy cell lymphoma and [Burketts] *Burkitts* lymphoma;

Column 6, lines 59–60:

[tumors of mesenchymal origin, including fibrosarcoma and rhabdomyoscarcoma;]

Column 6, lines 66–67:

tumors of mesenchymal origin, including fibrosarcoma, [rhabdomyoscaroma] *rhabdomyosarcoma*, and osteosarcoma; and

Column 7, lines 33–44:

Each of the compounds represented by formulae I and II may also be formulated or co-administered with other therapeutic agents that are selected for their particular usefulness in administering therapies [associates] *associated* with the aforementioned conditions. For example, each of the compounds of formulae I and II may be formulated with agents to prevent nausea, hypersensitivity, and gastric irritation, such as anti-emetics, and H_1 and H_2 antihistamines. The

above therapeutic agents, when employed in combination with the compound of formulae I or II, may be used in those amounts indicated in the Physicians' Desk Reference (PDR) or as otherwise determined by one of ordinary skill in the art.

Column 7, line 45 to column 8, line 10:

Furthermore, compounds of formulae I or II may be administered in combination with other anti-cancer and cytotoxic agents and treatments useful in the treatment of cancer or other proliferative diseases. Especially useful are anti-cancer and cytotoxic drug combinations wherein the second drug chosen acts in a different manner or different phase of the cell cycle, e.g., S phase, than the present compounds of formula I and II which exert their effects at the G_2-M phase. Example classes of anti-cancer and cytotoxic agents include, but are not limited to, alkylating agents, such as [nitorgen] *nitrogen* mustards, alkyl sulfonates, nitrosoureas, ethylenimines, and triazenes; antimetabolites, such as folate antagonists, purine analogues, and pyrimidine analogues; antibiotics, such as anthracyclines, bleomycins, mitomycin, dactinomycin, and plicamycin; enzymes, such as L-asparaginase; farnesyl-protein transferase inhibitors; hormonal agents, such as glucocorticoids, estrogens/antiestrogens, androgens/antiandrogens, progestins, and luteinizing hormone-releasing hormone anatagonists, octreotide acetate; microtubule-disruptor agents, such as ectenascidins or their analogs and derivatives; microtubule-stabilizing agents such as paclitaxel (Taxol®), docetaxel (Taxotere®); plant-derived products, such as vinca alkaloids, epipodophyllotoxins, taxanes; and topoisomerase inhibitors; prenyl-protein transferase inhibitors; and miscellaneous agents such as, hydroxyurea, procarbazine, mitotane, hexamethylmelamine, platinum coordination complexes such as cisplatin and carboplatin; and other agents used as anti-cancer and cytotoxic agents such as biological response modifiers, growth factors; immune modulators, and monoclonal antibodies. Compounds represented by formulae I and II may also be used in conjunction with radiation therapy.

Column 8, lines 11–27:

Representative examples of these classes of anti-cancer and cytotoxic agents include, but are not limited to, mechlorethamine [hydrochlordie] *hydrochloride*, cyclophosphamide, chlorambucil, melphalan, ifosfamide, busulfan, carmustin, lomustine, semustine, streptozocin, thiotepa, dacarbazine, methotrexate, thioguanine, mercaptopurine, fludarabine, pentastatin, cladribin, cytarabine, fluorouracil, doxorubicin hydrochloride, daunorubicin, idarubicin, bleomycin sulfate, mitomycin C, actinomycin D, safracins, saframycins, quinocarcins, discodermolides, vincristine, vinblastine, vinorelbine tartrate, etoposide, teniposide, paclitaxel, tamoxifen, estramustine, estramustine phosphate sodium, flutamide, buserelin, leuprolide, pteridines, diyneses, levamisole, aflacon, interferon, interleukins, aldesleukin, filgrastim, sargramostim, rituximab, BCG, tretinoin, irinotecan hydrochloride, betamethosone, gemcitabine hydrochloride, altretamine, and topoteca and any analogs or derivatives thereof.

Column 9, lines 33–43:

It has unexpectedly been found that the stability of the subject epothilone analogs can be significantly enhanced by carrying out the preparation of the solution at a temperature below ambient, preferably from about [5EC] *5° C.* to about [15EC] *15° C.*, more preferably about [5EC] *5° C.* Further, both the process of forming the solution and subsequent lyophilization are to be carried out in vessels such that the epothilone analogs are protected from exposure to light. It is

also beneficial to carry out the lyophilization in comparatively small batches so that the epothilone analogs are exposed to an aqueous medium for a minimum amount of time.

Column 9, lines 44–55:

The primary drying stage of lyophilization of the solution formed as described above is carried out at temperatures from about [−10EC to about −40EC] *−40° C. to about −10° C.*, preferably about [−25 EC] *−25° C.*, under high vacuum, ie., from about 50 millitorr to about 300 millitorr, preferably about 200 millitorr, for an extended period, i.e., from about 24 hours to about 96 hours, preferably about 48 hours. Lyophilization in this temperature range produces an amorphous product which is desirable for an intravenous preparation. Those of ordinary skill in the art will appreciate that conventional procedures, such as powder X-ray diffraction, can be utilized to confirm the amorphous nature of the lyophilized product.

Column 9, lines 56–62:

The residual solvents in the product are removed by a secondary drying stage that is carried out at comparatively low temperatures, i.e., from about [10 EC] *10° C.* to about [30 EC] *30° C.*, preferably about [25 EC] *25° C.*, under high vacuum, i.e., from about 50 millitorr to about 300 millitorr, preferably about 150 millitorr for an extended period, i.e., from about 24 hours to about 96 hours, preferably about 48 hours.

Column 10, lines 28–38:

The final dilution of the reconstituted epothilone analog in the formulation of the invention may be carried out with other preparations having similar utility, for example, 5% Dextrose Injection, Lactated Ringer's and Dextrose Injection, Sterile Water for Injection, and the like. However, because of its narrow pH range, pH 6.0 to 7.5, Lactated Ringer's Injection is preferred. Per 100 mL, Lactated Ringer's Injection contains Sodium Chloride USP 0.6 g, Sodium Lactate 0.31 g, Potassium chloride USP 0.03 g and [Calcium Chloride-2H20] *Calcium Chloride·2H_2O* USP 0.02 g. The osmolarity is 275 mOsmol/L, which is very close to isotonicity.

Column 11, lines 40–43:

One of ordinary skill in the art would readily know how to convert doses from mg/kg to [mg/m2] *mg/m^2* given either or both the height and or weight of the patient (See, e.g., http://www.fda.gov/cder/cancer/animalframe.htm).

Column 13, line 66 to column 14, line 11:

[1S-[1R*,3R*(E),7R*,10S*,11R*,12R*,16S*]]-7,11-Dihydroxy-8,8,10,12,16-pentamethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-4-aza-17-oxabicyclo[14.1.0]heptadecane-5,9-dione, 9.86 g, was wetted/partially dissolved with 600 mL of a 9:1 mixture of tertiary butanol and Water for Injection USP which had been pre-cooled to [5 EC] *5° C.* Once the drug powder had become completely wetted, dissolution was completed by the addition of 600 mL of a 1:9 mixture of tertiary butanol and Water for Injection and 766 mL of a 1:1 mixture of tertiary butanol and Water for Injection which likewise had been pre-cooled to [5 EC] *5° C.* thereby making the final solution a 1:1 mixture. The dissolution was carried out under protection from light.

Column 14, lines 12–20:

The solution formed above was promptly lyophilized in a Virtis lNOTOP lyophilizer at [−16 EC] *−16° C.* under light [protectant] *protected* conditions over a period of 48 hours. The resultant lyophilized product (lyophile) was then further dried at [15 EC] *15° C.* under high vacuum for 48 hours. No detectable degradation of the drug was observed during these procedures. The lyophile was packaged under sterile

conditions into 30 mL vials, each containing 10 mg of drug and standard excess to allow for vial/needle/syringe loss.

Column 14, lines 31–45:

A total of 24 cancer patients (12 male and 12 female) received compound 11 by 1V administration to evaluate the maximum tolerated dose (MTD), the dose limiting toxicity (DLT), the **[**pharmacokinitics**]** *pharmacokinetics* and pharmacodynamics, and to evaluate the anti-tumor activity of compound 11. The median age (range) of the patients was 57 (34–74). 5 patients had breast cancer, 5 patients had head and neck cancer, 2 patients had sarcoma, 2 patients had colorectal cancer, 2 patients had UPT cancer, 2 patients had melanoma, 2 patients had cancer of the esophagus, 1 patient had renal cancer, 1 patient had cervical cancer, 1 patient had thyroid cancer, and 1 patient had anal cancer. 21 patients had received prior chemotherapy (18 patients received neurotoxic agents and 18 patients received radiotherapy). The median number of prior chemotherapy lines including adjuvant (range) was 2 (1–3).

Column 15, lines 34–45:

Samples were analyzed by adding an internal standard to 0.2 mL of plasma sample, precipitating with acetone, and then extracting the supernatant with 1-chlorobutane. The organic layer was removed and evaporated to dryness. The residue was reconstituted and injected into the LC/MS/MS system. Chromatographic separation was achieved, isocratically, on a YMC ODS-AQ column (4.6×50 mm, 3:m) with a mobile phase of acetonitrile:0.01M ammonium acetate, pH 5.0 (65:35). Detection was by negative electrospray tandem mass spectrometry. The standard curve, which ranged from 2 to 500 ng/mL for all analytes**[**and**]**, was fitted to a 1/x weighted quadratic regression model.

Column 16, lines 22–30:

lmmediately after blood collection, the Vacutainer tubes were inverted several times to ensure mixing with the anticoagulant and then immediately placed on crushed ice. Within 30 minutes of collection, samples were centrifuged for 5 minutes at approximately 2000×g and 0 to **[**5 EC**]** *5° C.* The plasma was then transferred to separate pre-labeled screw-capped polypropylene tubes and stored at **[**−70 EC**]** *−70° C.* until bioanalysis. Plasma concentrations on Compound 11 were analyzed using the LC/MS/MS assay.

AS A RESULT OF REEXAMlNATlON, 1T HAS BEEN DETERMlNED THAT:

The patentability of claims **15**–**23**, **63**–**70** and **72**–**77** is confirmed.

Claims **60**, **62** and **71** are cancelled.

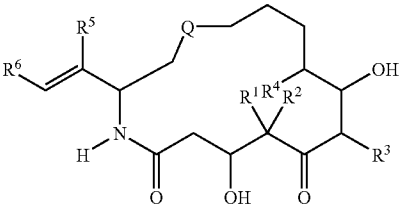
Claims **1**, **7**–**10**, **24**–**26**, **30**, **32**, **34**, **35**, **44**, **49**, **52**, **56**, **58**, **78**, **79**, **82**, **86**, **88**, **93**–**95** and **101** are determined to be patentable as amended.

Claims **2**–**6**, **11**–**14**, **27**–**29**, **31**, **33**, **36**–**43**, **45**–**48**, **50**, **51**, **53**–**55**, **57**, **59**, **61**, **80**, **81**, **83**–**85**, **87**, **89**–**92**, **96**–**100** and **102**–**105**, dependent on an amended claim, are determined to be patentable.

New claims **106**–**110** are added and determined to be patentable.

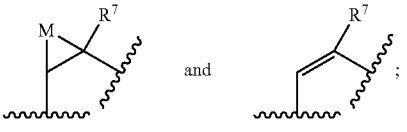
1. A process for formulating, for parenteral administration, an epothilone analog represented by formula 1:

I



wherein:

Q is selected from the group consisting of:



;

and

M is selected from the group consisting of oxygen, sulfur, NR^8, and CR^9R^{10};

each R^1, R^2, R^3, R^4, R^5, R^7, R^{11}, R^{12}, R^{13}, R^{14}, and R^{15} is, independently, selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, substituted aryl and heterocyclo, and wherein **[**R^1, and R^2,**]** *R^1 and R^2* are alkyl, they can be joined to form cycloalkyl;

R^6**[**,**]** is selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, cycloalkyl, heterocyclo and substituted heterocyclo;

R^8 is selected from the group consisting of hydrogen, alkyl, substituted alkyl, **[**R^{14}, C═O**]** *$R^{11}C$═O*, $R^{12}OC$═O and $R^{13}SO_2$; and

each R^9 and R^{10} is, independently, selected from the group consisting of hydrogen, halogen, alkyl, substituted alkyl, aryl, heterocyclo, hydroxy, $R^{14}C$═O, and $R^{15}OC$═O;

and any **[**salts,**]** solvates, or hydrates thereof, comprising the following steps carried out under protection from light:

a) dissolving said epothilone analog in a mixture of at least about 50% by volume tertiary-butanol in water to form a solution;

b) performing primary drying of said solution at a temperature of from about **[**−10° C. to about −40° C.**]** *−40° C. to about −10° C.* under high vacuum of from about 50 millitorr to about 300 millitorr for from about 24 hours to about 96 hours to form a dried product;

c) performing secondary drying of the resultant dried product at a temperature of from about **[**10° C.**]** *10° C.* to about **[**30° C.**]** *30° C.* under high vacuum of from about 50 millitorr to about 300 millitorr for from 24 hours to about 96 hours to provide a lyophilized product; and

d) packaging said lyophilized product in a first vial in combination with a second vial containing a sufficient quantity of an equal mixture by volume of a suitable nonionic surfactant and anhydrous ethanol to effect solution thereof.

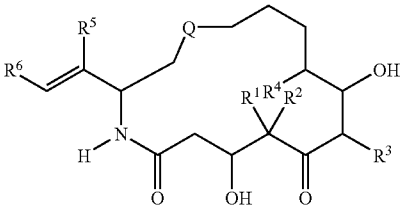
7. The process of claim **1** wherein said primary drying in step b) is carried out at a temperature of about **[**−25° C.**]** *−25° C.* and a pressure of about 200 millitorr for about 48 hours.

8. The process of claim **2** wherein said primary drying in step b) is carried out at a temperature of about **[**−25° C.**]** *−25° C.* and a pressure of about 200 millitorr for about 48 hours.

9. The process of claim **1** wherein said secondary drying in step c) is carried out at a temperature of about **[**25° C.**]** *25° C.* and a pressure of about 150 millitorr for about 48 hours.

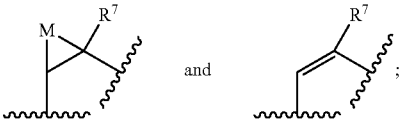
10. The process of claim **2** wherein said secondary drying in step c) is carried out at a temperature of about **[**25° C.**]** *25° C.* and a pressure of about 150 millitorr for about 48 hours.

24. A method for treating a patient in need of treatment with an epothilone analog represented *by* formula 1:



wherein:

Q is selected from the group consisting of **[**M,**]**



M is selected from the group consisting of oxygen, sulfur, NR^8, and CR^9R^{10};

each R^1, R^2, R^3, R^4, R^5, R^7, R^{11}, R^{12}, R^{13}, R^{14}, and R^{15} is, independently, selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, substituted aryl and heterocyclo, and wherein R^1 and R^2 are alkyl, they can be joined to form a cycloalkyl;

R^6 is selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, cycloalkyl, heterocyclo and substituted heterocyclo;

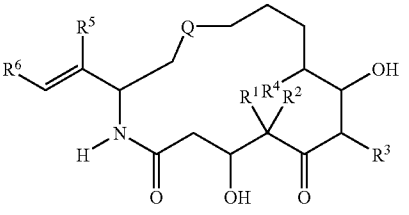
R^8 is selected from the group consisting of hydrogen, alkyl, substituted alkyl, $R^{11}C{=}O$, $R^{12}OC{=}O$ and $R^{13}SO_2$; and

each R^9 and R^{10} is, independently, selected from the group consisting of hydrogen, halogen, alkyl, substituted alkyl, aryl, heterocyclo, hydroxy, $R^{14}C{=}O$, and $R^{15}OC{=}O$;

and any **[**salts,**]** solvates, or hydrates thereof, comprising administering to said patient, by intravenous injection or infusion, an effective amount of a pharmaceutical composition *prepared according to the method* of claim **18**.

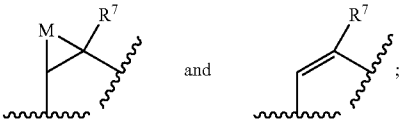
25. A method for treating a patient in need of treatment with an epothilone analog represented formula 1:

I



wherein:

Q is selected from the group consisting of



M is selected from the group consisting of oxygen, sulfur, NR^8, and CR^9R^{10};

each R^1, R^2, R^3, R^4, R^5, R^7, R^{11}, R^{12}, R^{13}, R^{14}, and R^{15} is independently, selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, substituted aryl and heterocyclo, and wherein **[**R'**]** R^1 and R^2 are alkyl, they can be joined to form a cycloalkyl; and

R^6 is selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, cycloalkyl, heterocyclo and substituted heterocyclo;

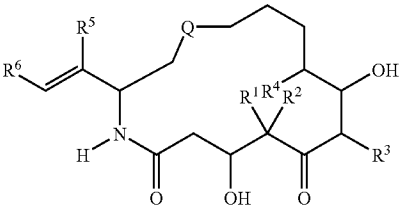
R^8 is selected from the group consisting of hydrogen, alkyl, substituted alkyl, **[**R^{11}, C═O**]** $R^{11}C{=}O$, $R^{12}OC{=}O$ and $R^{13}SO_2$; and

each R^9 and R^{10} is, independently, selected from the group consisting of hydrogen, halogen, alkyl, substituted alkyl, aryl, heterocyclo, hydroxy, $R^{14}C{=}O$, and $R^{15}OC{=}O$;

and any **[**salts,**]** solvates, or hydrates thereof, comprising administering to said patient, by intravenous injection or infusion, an effective amount of a pharmaceutical composition *prepared according to the method* of claim **19**.

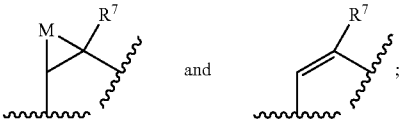
26. A method for treating a patient in need of treatment with an epothilone analog represented formula 1:

I



wherein:

Q is selected from the group consisting of



M is selected from the group consisting of oxygen, sulfur, NR^8, and CR^9R^{10};

each [R'] R^1, R^2, R^3, R^4, R^5, R^7, R^{11}, R^{12}, R^{13}, R^{14}, and R^{15} is, independently, selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, substituted aryl and heterocyclo, and wherein R^1 and R^2 are alkyl, they can be joined to form a cycloalkyl;

R^6 is selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, cycloalkyl, heterocyclo and substituted heterocyclo;

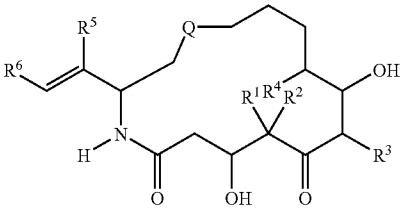
R^8 is selected from the group consisting of hydrogen, alkyl, substituted alkyl, $R^{11}C{=}O$, $R^{12}OC{=}O$ and $R^{13}SO_2$; and

each R^9 and R^{10} is, independently, selected from the group consisting of hydrogen, halogen, alkyl, substituted alkyl, aryl, heterocyclo, hydroxy, [$R^{14C=O}$] $R^{14}C{=}O$, and $R^{15}OC{=}O$;

and any [salts,] solvates, or hydrates thereof, comprising administering to said patient, by intravenous injection or infusion, an effective amount of a pharmaceutical composition *prepared according to the method* of claim **20**.

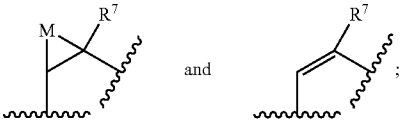
30. A pharmaceutical composition suitable for parenteral administration comprising in lyophilized form a compound represented by formula 1:

I



wherein:

Q is selected from the group consisting of



M is selected from the group consisting of oxygen, sulfur, NR^8, and CR^9R^{10};

each [R'] R^1, R^2, R^3, R^4, R^5, R^7, R^{11}, R^{12}, R^{13}, R^{14} and R^{15} *is*, independently, selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, substituted aryl and heterocyclo, and wherein [R'] R^1 and R^2 are alkyl, they can be joined to form a cycloalkyl;

R^6 is selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, cycloalkyl, heterocyclo and substituted heterocyclo;

R^8 is selected from the group consisting of hydrogen, alkyl, substituted alkyl, $R^{11}C{=}O$, [$R^2OC{=}O$] $R^{12}OC{=}O$ and $R^{13}SO_2$; and

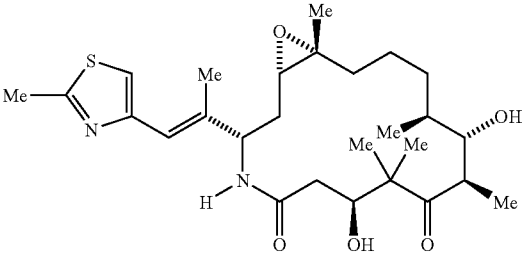
each R^9 and R^{10} is, independently, selected from the group consisting of hydrogen, halogen, alkyl, substituted alkyl, aryl, heterocyclo, hydroxy, $R^{14}CO{=}O$, and $R^{15}OC{=}O$;

and any [salts,] solvates, or hydrates thereof;
dehydrated alcohol;
and a [non-ionic] *nonionic* surfactant.

32. The composition of claim **30**, wherein the surfactant is Cremophor EL® *surfactant*.

34. A pharmaceutical composition suitable for parenteral administration comprising a compound represented by formula 1l:

II



and any [salts,] solvates, or hydrates thereof;
dehydrated alcohol; and
a [non-ionic] *nonionic* surfactant.

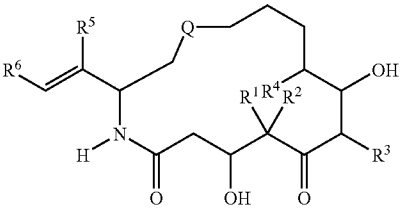
35. A method of treating cancer in a patient comprising intravenously administering to said patient a therapeutically effective amount of the pharmaceutical [formulation] *composition* of claim **30** diluted in a parenteral diluent.

44. The method of claim **43**, wherein the one or more additional agents is an [H1 or H2] H_1 *or* H_2 antihistamine.

49. A method of treating cancer in a patient previously experiencing neurotoxicity comprising intravenously administering to said patient a therapeutically effective amount of the pharmaceutical [formulation] *composition* of claim **30** diluted in a parenteral diluent as a weekly infusion, wherein the total dose of the compound of formula 1 is less than about 200 mg/m^2.

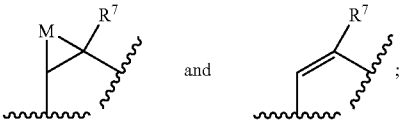
52. A method of treating cancer while reducing or avoiding neurotoxicity which comprises intravenously administering a therapeutically effective amount of compound represented by formula 1:

I



wherein:

Q is selected from the group consisting of



M is selected from the group consisting of oxygen, sulfur, NR^8 and CR^9, R^{10},

each R^1, R^2, R^3, R^4, R^5, R^7, R^{11}, R^{12}, R^{13}, R^{14} and R^{15} is, independently, selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, substituted aryl and heterocyclo, and wherein [R'] R^1 and R^2 are alkyl, they can be joined to form a cycloalkyl;

R^6 is selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, cycloalkyl, heterocyclo and substituted heterocyclo;

R^8 is selected from the group consisting of hydrogen, alkyl, substituted alkyl, $R^{11}C{=}O$, $R^{12}OC{=}O$ and $R^{12}SO_2$ and

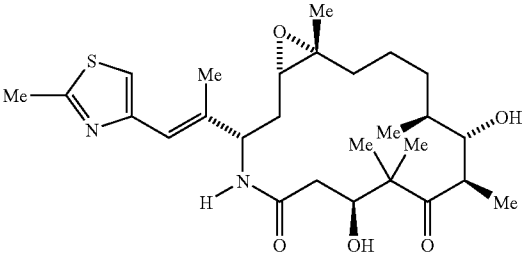
each R^9 and R^{10} is, independently, selected from the group consisting of hydrogen, halogen, alkyl, substituted alkyl, aryl, heterocyclo, hydroxy, $R^{14}C{=}O$, and $R^{15}OC{=}O$;

and any [salts,] solvates, or hydrates thereof;

over a period of one (1) hour to a patient in need thereof.

56. A method of treating cancer while reducing or avoiding neurotoxicity which comprises intravenously infusing a therapeutically effective amount of compound represented by formula [I] *II*:

II



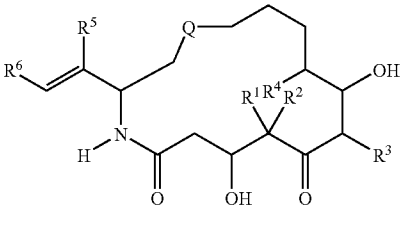
over a period of one (1) hour to a patient in need thereof.

58. A method of treating cancer in a human patient in need thereof with a synthetic or semi-synthetic epothilone [analogue] *analog* that is active against cancer which comprises a four (4) week dosing cycle wherein said cycle comprises three weeks of weekly intravenous administration and one week of oral administration of said epothilone [analogue] *analog*.

78. The pharmaceutical preparation of claim **15**, wherein the quantity of solvent is an amount such that when the solvent is combined with the lyophilized epothilone *analog* the resulting solution contains from about 2 mg/mL to about 4 mg/mL of said analog.

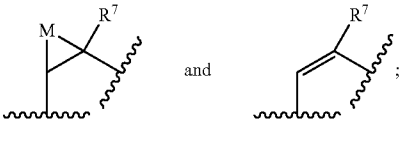
79. A method of treating cancer in a patient comprising intravenously administering to said patient daily for 3 days or daily for 5 days a therapeutically effective amount of a compound represented by formula 1:

I



wherein:

Q is selected from the group consisting of



M is selected from the group consisting of oxygen, sulfur, [NR] *NR*^{8}, and CR^9R^{10};

each R^1, R^2, R^3, R^4, R^5, R^7, R^{11}, R^{12}, R^{13}, R^{14}, and R^{15}[,] is, independently, selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, substituted aryl and heterocyclo, and wherein R^1 and R^2 are alkyl, they can be joined to form a cycloalkyl;

R^6 is selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, cycloalkyl, heterocyclo and substituted heterocyclo;

R^8 is selected from the group consisting of hydrogen, alkyl, substituted alkyl, $R^{11}C{=}O$, $R^{12}OC{=}O$ and $R^{13}SO_2$; and

each R^9 and R^{10} is, independently, selected from the group consisting of hydrogen, halogen, alkyl, substituted alkyl, aryl, heterocyclo, hydroxy, $R^{14}C{=}O$, and $R^{15}OC{=}O$;

and any [salts,] solvates, or hydrates thereof.

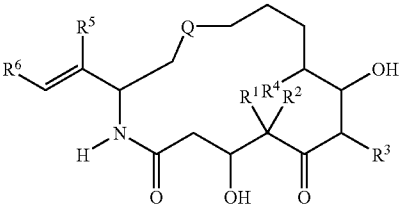
82. The method of claim **81**, wherein the compound of formula 1 is administered [in] *at* a dose of about 0.05 mg/kg to 200 mg/kg.

86. The method of claim **79**, wherein the IV infusion is administered over a [perioe] *period* of about 1 hour.

88. The method of claim **86**, wherein the one or more additional therapeutic agents is an [H^1, or H^2,] H_1 *or* H_2 antihistamine.

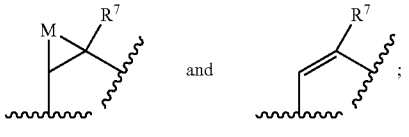
93. A method of treating cancer in a patient comprising intravenously administering to said patient every week or every 3 weeks a therapeutically effective amount of a compound represented by formula 1:

I



wherein:

Q is selected from the group consisting of



M is selected from the group consisting of oxygen, sulfur, [NR] *NR*^{8}, and CR^9R^{10};

each R^1, R^2, R^3, R^4, R^5, R^7, R^{11}, R^{12}, R^{13}, R^{14}, and R^{15}[,] is, independently, selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, substituted aryl and heterocyclo, and wherein R^1 and R^2 are alkyl, they can be joined to form a cycloalkyl;

R^6 is selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, cycloalkyl, heterocyclo and substituted heterocyclo;

R^8 is selected from the group consisting of hydrogen, alkyl, substituted alkyl, $R^{11}C{=}O$, $R^{12}OC{=}O$ and $R^{13}SO_2$; and

each R^9 and R^{10} is, independently, selected from the group consisting of hydrogen, halogen, alkyl, substituted alkyl, aryl, heterocyclo, hydroxy, $R^{14}C{=}O$, and $R^{15}OC{=}O$;

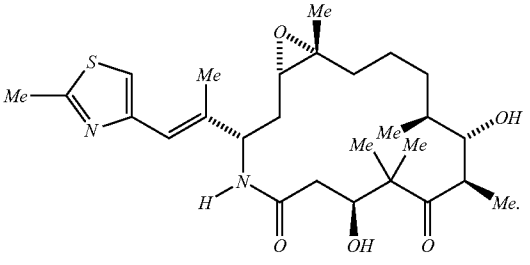
and any [salts,] solvates, or hydrates thereof.

94. The method of *claim* **93**, wherein the compound of formula 1 is administered every week.

95. The method of *claim* **93**, wherein the compound of formula 1 is administered every 3 weeks.

101. The method of claim **100**, wherein the one or more additional therapeutic agents is an [H^1, or H^2,] H_1 *or* H_2 antihistamine.

106. The method of claim 95, wherein the compound of formula I is:



107. The method of claim 106, wherein the compound of formula I is administered in a dose of about 0.05 mg/kg to 200 mg/kg.

108. The method of claim 107, wherein the compound of formula I is administered over a period of about 10 minutes to about 3 hours.

109. The method of claim 107, wherein the compound of formula I is administered in a dose of about 30 mg/kg to 65 mg/kg.

110. The method of claim 109, wherein the compound of formula I is administered over a period of about 3 hours.

* * * * *

Exhibit B

US007022330B2

(12) **United States Patent**
Bandyopadhyay et al.

(10) **Patent No.: US 7,022,330 B2**
(45) **Date of Patent: Apr. 4, 2006**

(54) **PARENTERAL FORMULATION FOR EPOTHILONE ANALOGS**

(75) Inventors: **Rebanta Bandyopadhyay**, Portage, MI (US); **Timothy M. Malloy**, Yardley, PA (US); **Andrea Panaggio**, West Windsor, NJ (US); **Krishnaswamy Srinivas Raghavan**, Cranbury, NJ (US); **Sailesh Amilal Varia**, Princeton Junction, NJ (US)

(73) Assignee: **Bristol-Myers Squibb Company**, Princeton, NJ (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 147 days.

(21) Appl. No.: **10/051,727**

(22) Filed: **Jan. 17, 2002**

(65) **Prior Publication Data**

US 2002/0143038 A1 Oct. 3, 2002

Related U.S. Application Data

(60) Provisional application No. 60/264,228, filed on Jan. 25, 2001.

(51) **Int. Cl.**
A61K 9/00 (2006.01)

(52) **U.S. Cl.** **424/400**; 424/422; 514/365; 514/37; 514/183; 548/146

(58) **Field of Classification Search** 424/400, 424/422; 514/183, 37, 365; 548/146
See application file for complete search history.

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Primary Examiner—Gollamudi S. Kishore
Assistant Examiner—Lakshmi Channavajjala
(74) *Attorney, Agent, or Firm*—Anastasia P. Winslow; Rena Patel

(57) **ABSTRACT**

A process for formulating certain epothilone analogs for parenteral administration is disclosed wherein the analog is dissolved in a mixture of at least 50% by volume tertiary-butanol in water, the mixture is lyophilized, the resulting lyophilized product is packaged in one vial with a sufficient amount of solvent comprising anhydrous ethanol and a suitable nonionic surfactant in a second vial. All steps are carried out with protection from light. In use, the contents of the second or diluent vial are added to the lyophilized product and mixed to constitute the epothilone analog and the resulting solution is diluted with a suitable diluent to produce a solution for intravenous injection containing the epothilone analog in a concentration of from about 0.1 mg/mL to about 0.9 mg/mL. A preferred surfactant is polyethoxylated castor oil and a preferred diluent is Lactated Ringer's Injection.

36 Claims, No Drawings

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PARENTERAL FORMULATION FOR EPOTHILONE ANALOGS

CROSS-REFERENCE TO RELATED APPLICATION

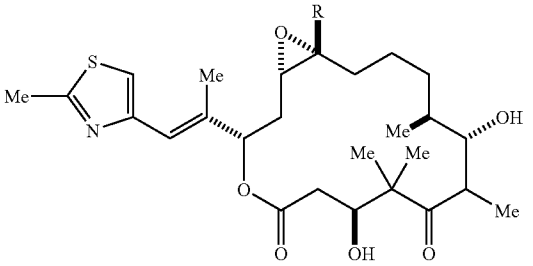
This application claims priority from provisional application Ser. No. 60/264,228, filed Jan. 25, 2001, incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

The present invention relates to an improved formulation for parenteral administration of certain epothilone analogs that are characterized by enhanced clinical efficacy.

BACKGROUND OF THE INVENTION

Epothilones are macrolide compounds having utility in the pharmaceutical field. For example, Epothilones A and B having the structures:



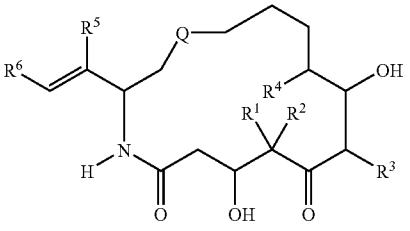
Epothilone A R = H

Epothilone B R = Me

may be found to exert microtubule-stabilizing effects similar to paclitaxel (TAXOL®) and hence cytotoxic activity against rapidly proliferating cells, such as, tumor cells or other hyperproliferative cellular disease, see Hofle et al., *Angew. Chem. Int. Ed. Engl.*, Vol. 35, No. 13/14, 1567–1569 (1996); WO 93/10121 published May 27, 1993; and WO 97/19086 published May 29, 1997.

Derivatives and analogs of Epothilones A and B have been synthesized and may be used to treat a variety of cancers and other abnormal proliferative diseases. Such analogs are disclosed in Hofle et al., Id.; Nicolaou et al., *Angew Chem. Int. Ed. Engl.*, Vol. 36, No. 19, 2097–2103 (1997); and Su et al., *Angew Chem. Int. Ed. Engl.*, Vol. 36, No. 19, 2093–2097 (1997).

Analogs of the epothilones that have been found to have advantageous activity are represented by formula I:



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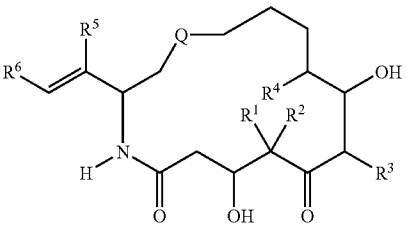
wherein the various symbols are as defined below. While these compounds possess significant therapeutic properties, they also present difficulties to those skilled in the art of pharmaceutical compounding, as a result of certain properties, as will be detailed hereinbelow. In accordance with the present invention, a formulation has been found whereby the epothilone analogs described above can be safely dispensed and administered via injection, without appreciable loss of potency.

SUMMARY OF THE INVENTION

The present invention describes a formulation and the preparation thereof for epothilone analogs represented by formula I.

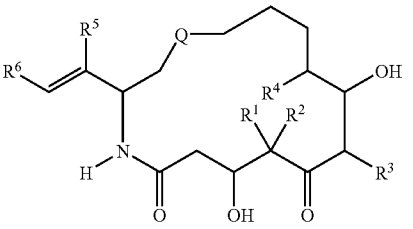


I

wherein the various symbols are as defined below. In the formulations of the present invention, the epothilone analog is initially solubilized with a mixture of tertiary-butanol and water and then lyophilized under optimized conditions. The lyophilized drug is reconstituted first with a mixture of a polyethoxylated castor oil surfactant and anhydrous ethanol, and thereafter diluted with Lactated Ringer's Injection to a concentration appropriate for administration.

DETAILED DESCRIPTION OF THE INVENTION

The process of the present invention provides an advantageous formulation for the administration of epothilone analogs represented by formula I:



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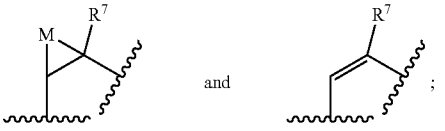
As used in formula I and throughout the specification, Q is selected from the group consisting of:



and ;

M is selected from the group consisting of oxygen, sulfur, NR^8, and CR^9R^{10};

each R^1, R^2, R^3, R^4, R^5, R^7, R^{11}, R^{12}, R^{13}, R^{14} and R^{15} is, in independently, selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, substituted aryl and heterocyclo, and wherein R^1 and R^2 are alkyl, they can be joined to form cycloalkyl;

R^6 is selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, cycloalkyl, heterocyclo and substituted heterocyclo;

R^8 is selected from the group consisting of hydrogen, alkyl, substituted alkyl, $R^{11}C{=}O$, $R^{12}OC{=}O$ and $R^{13}SO_2$; and

each R^9 and R^{10} is, independently, selected from the group consisting of hydrogen, halogen, alkyl, substituted alkyl, aryl, heterocyclo, hydroxy, $R^{14}C{=}O$, and $R^{15}C{=}O$.

The following are definitions of various terms used herein to describe the present invention. These definitions apply to the terms as they are used throughout this specification, unless otherwise limited in specific instances, either individually or as part of a larger group.

The term "alkyl" refers to optionally substituted straight- or branched-chain saturated hydrocarbon groups having from 1 to about 20 carbon atoms, preferably from 1 to about 7 carbon atoms. The expression "lower alkyl" refers to optionally substituted alkyl groups having from 1 to about 4 carbon atoms.

The term "substituted alkyl" refers to an alkyl group substituted by, for example, one to four substituents, such as, halo, trifluoromethyl, trifluoromethoxy, hydroxy, alkoxy, cycloalkyoxy, heterocylooxy, oxo, alkanoyl, aryl, aryloxy, aralkyl, alkanoyloxy, amino, alkylamino, arylamino, aralkylamino, cycloalkylamino, heterocycloamino, disubstituted amino in which the two substituents on the amino group are selected from alkyl, aryl, aralkyl, alkanoylamino, aroylamino, aralkanoylamino, substituted alkanoylamino, substituted arylamino, substituted aralkanoylamino, thiol, alkylthio, arylthio, aralkylthio, cycloalkylthio, heterocyclothio, alkylthiono, arylthiono, aralkylthiono, alkylsulfonyl, arylsulfonyl, aralkylsulfonyl, sulfonamido (e.g., SO_2NH_2), substituted sulfonamido, nitro, cyano, carboxy, carbamyl (e.g., $CONH_2$), substituted carbamyl (e.g., CONH alkyl, CONH aryl, CONH aralkyl or instances where there are two substituents on the nitrogen selected from alkyl, aryl or aralkyl), alkoxycarbonyl, aryl, substituted aryl, guanidino and heterocyclos, such as, indolyl, imidazolyl, furyl, thienyl, thiazolyl, pyrrolidyl, pyridyl, pyrimidyl and the like. Wherein, as noted above, the substituents themselves are further substituted, such further substituents are selected from the group consisting of halogen, alkyl, alkoxy, aryl and aralkyl. The definitions given herein for alkyl and substituted alkyl apply as well to the alkyl portion of alkoxy groups.

The term "halogen" or "halo" refers to fluorine, chlorine, bromine and iodine.

The term "ring system" refers to an optionally substituted ring system containing one to three rings and at least one carbon to carbon double bond in at least one ring. Exemplary ring systems include, but are not limited to, an aryl or a partially or fully unsaturated heterocyclic ring system, which may be optionally substituted.

The term "aryl" refers to monocyclic or bicyclic aromatic hydrocarbon groups having from about 6 to about 12 carbon atoms in the ring portion, for example, phenyl, naphthyl, biphenyl and diphenyl groups, each of which may be substituted.

The term "aralkyl" refers to an aryl group bonded to a larger entity through an alkyl group, for example, a benzyl group.

The term "substituted aryl" refers to an aryl group substituted by, for example, one to four substituents such as alkyl; substituted alkyl, halo, trifluoromethyl, trifluoromethoxy, hydroxy, alkoxy, cycloalkyloxy, heterocyclooxy, alkanoyl, alkanoyloxy, amino, alkylamino, dialkylamino, aralkylamino, cycloalkylamino, heterocycloamino, alkanoylamino, thiol, alkylthio, cycloalkylthio, heterocyclothio, ureido, nitro, cyano, carboxy, carboxyalkyl, carbamyl, alkoxycarbonyl, alkylthiono, arylthiono, alkysulfonyl, sulfonamido, aryloxy and the like. The substituent may be further substituted by one or more members selected from the group consisting of halo, hydroxy, alkyl, alkoxy, aryl, substituted alkyl, substituted aryl and aralkyl.

The term "cycloalkyl" refers to optionally substituted saturated cyclic hydrocarbon ring systems, preferably containing 1 to 3 rings and 3 to 7 carbons per ring, which may be further fused with an unsaturated C_3–C_7 carbocyclic ring. Exemplary groups include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, cyclodecyl, cyclododecyl, and adamantyl. Exemplary substituents include one or more alkyl groups as described above, or one or more of the groups described above as substituents for alkyl groups.

The terms "heterocycle", "heterocyclic" and "heterocyclo" refer to an optionally substituted, unsaturated, partially saturated, or fully saturated, aromatic or nonaromatic cyclic group, for example, which is a 4 to 7 membered monocyclic, 7 to 11 membered bicyclic, or 10 to 15 membered tricyclic ring system, which has at least one heteroatom in at least one carbon atom-containing ring. Each ring of the heterocyclic group containing a heteroatom may have 1, 2 or 3 heteroatoms selected from nitrogen atoms, oxygen atoms and sulfur atoms, where the nitrogen and sulfur heteroatoms may also optionally be oxidized and the nitrogen heteroatoms may also optionally be quaternized. The heterocyclic group may be attached at any heteroatom or carbon atom.

Exemplary monocyclic heterocyclic groups include pyrrolidinyl, pyrrolyl, indolyl, pyrazolyl, oxetanyl, pyrazolinyl, imidazolyl, imidazolinyl, imidazolidinyl, oxazolyl, oxazolidinyl, isoxazolinyl, isoxazolyl, thiazolyl, thiadiazolyl, thiazolidinyl, isothiazolyl, isothiazolidinyl, furyl, tetrahydrofuryl, thienyl, oxadiazolyl, piperidinyl, piperazinyl, 2-oxopiperazinyl, 2-oxopiperidinyl, 2-oxopyrrolidinyl, 2-oxazepinyl, azepinyl, 4-piperidonyl, pyridyl, N-oxo-pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, tetrahydropyranyl, tetrahydrothiopyranyl, tetrahydrothiopyranyl sulfone, morpholinyl, thiomorpholinyl, thiomorpholinyl sulfoxide, thiomorpholinyl sulfone, 1,3-dioxolane and tetrahydro-1,1-dioxothienyl, dioxanyl, isothiazolidinyl, thietanyl, thiiranyl, triazinyl, and triazolyl, and the like.

Exemplary bicyclic heterocyclic groups include benzothiazolyl, benzoxazolyl, benzothienyl, quinuclidinyl, quinolinyl, quinolinyl-N-oxide, tetrahydroisoquinolinyl, isoquinolinyl, benzimidazolyl, benzopyranyl, indolizinyl, benzofuryl, chromonyl, coumarinyl, cinnolinyl, quinoxalinyl, indazolyl, pyrrolopyridyl, furopyridinyl (such as furo[2,3-c]pyridinyl, furo[3,1-b]pyridinyl] or furo[2,3-b]pyridinyl), dihydroisoindolyl, dihydroquinazolinyl (such as 3,4-dihydro-4-oxo-quinazolinyl), benzisothiazolyl, benzisoxazolyl, benzodiazinyl, benzofurazanyl, benzothiopyranyl, benzotriazolyl, benzpyrazolyl, dihydrobenzofuryl, dihydrobenzothienyl, dihydrobenzothiopyranyl, dihydrobenzothiopyranyl sulfone, dihydrobenzopyranyl, indolinyl, isochromanyl, isoindolinyl, naphthyridinyl, phthalazinyl, piperonyl, purinyl, pyridopyridyl, quinazolinyl, tetrahydroquinolinyl, thienofuryl, thienopyridyl, thienothienyl, and the like.

Exemplary substituents for the terms "ring system," "heterocycle," "heterocyclic," and "heterocyclo" include one or more substituent groups as described above for substituted alkyl or substituted aryl, and smaller heterocyclos, such as, epoxides, aziridines and the like.

The term "alkanoyl" refers to —C(O)-alkyl.

The term "substituted alkanoyl" refers to —C(O)-substituted alkyl.

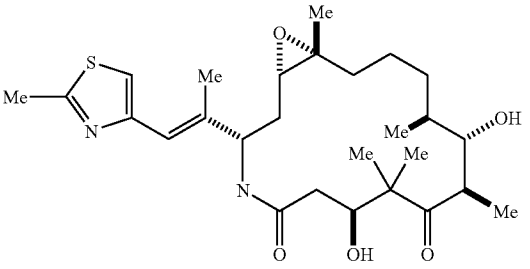
The term "heteroatoms" shall include oxygen, sulfur and nitrogen.

The compounds represented by formula I form salts with a variety of organic and inorganic acids. Such salts include those formed with hydrogen chloride, hydrogen bromide, methanesulfonic acid, hydroxyethanesulfonic acid, sulfuric acid, acetic acid, trifluoroacetic acid, maleic acid, benzenesulfonic acid, toluenesulfonic acid and various others as are recognized by those of ordinary skill in the art of pharmaceutical compounding. Such salts are formed by reacting a compound represented by formula I in an equivalent amount of the acid in a medium in which the salt precipitates or in an aqueous medium followed by evaporation.

In addition, zwitterions ("inner salts") can be formed and are included within the term salts as used herein.

A particularly preferred epothilone analog within those represented by formula I is [1S-[1R*,3R*(E),7R*,10S*,11R*,12R*,16S*]]-7,11-Dihydroxy-8,8,10,12,16-pentamethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-4-aza-7-oxabicyclo[14.1.0]heptadecane-5,9-dione represented by formula II

II



The compounds represented by formulae I and II above and their preparation are described in U.S. patent application Ser. No. 09/170,582, filed Oct. 13, 1998, the disclosure of which is incorporated herein by reference. The compounds represented by formulae I and II above may exist as multiple optical, geometric, and stereoisomers. While the compounds shown herein are depicted for one optical orientation, included within the present invention are all isomers and mixtures thereof.

The compounds represented by formulae I and II above are microtubule-stabilizing agents. They are thus useful in the treatment of a variety of cancers and other proliferative diseases including, but not limited to, the following:

carcinoma, including that of the bladder, breast, colon, kidney, liver, lung, ovary, pancreas, stomach, cervix, thyroid and skin, including squamous cell carcinoma;

hematopoietic tumors of lymphoid lineage, including leukemia, acute lymphocytic leukemia, acute lymphoblastic leukemia, B-cell lymphoma, T-cell lymphoma, Hodgkins lymphoma, non-Hodgkins lymphoma, hairy cell lymphoma and Burketts lymphoma;

hematopoietic tumors of myeloid lineage, including acute and chronic myelogenous leukemias and promyelocytic leukemia;

tumors of mesenchymal origin, including fibrosarcoma and rhabdomyoscarcoma;

other tumors, including melanoma, seminoma, teratocarcinoma, neuroblastoma and glioma;

tumors of the central and peripheral nervous system, including astrocytoma, neuroblastoma, glioma, and schwannomas;

tumors of mesenchymal origin, including fibrosarcoma, rhabdomyoscaroma, and osteosarcoma; and

other tumors, including melanoma, xeroderma pigmentosum, keratoacanthoma, seminoma, thyroid follicular cancer and teratocarcinoma.

The compounds represented by formulae I and II above will also inhibit angiogenesis, thereby affecting the growth of tumors and providing treatment of tumors and tumor-related disorders. Such anti-angiogenesis properties of the compounds represented by formulae I and II will also be useful in the treatment of other conditions responsive to anti-angiogenesis agents including, but not limited to, certain forms of blindness related to retinal vascularization, arthritis, especially inflammatory arthritis, multiple sclerosis, restinosis and psoriasis.

Compounds represented by formulae I and II will induce or inhibit apoptosis, a physiological cell death process critical for normal development and homeostasis. Alterations of apoptotic pathways contribute to the pathogenesis of a variety of human diseases. Compounds represented by formulae I and II, as modulators of apoptosis, will be useful in the treatment of a variety of human diseases with aberrations in apoptosis including, but not limited to, cancer and precancerous lesions, immune response related diseases, viral infections, degenerative diseases of the musculoskeletal system and kidney disease.

Each of the compounds represented by formulae I and II may also be formulated or co-administered with other therapeutic agents that are selected for their particular usefulness in administering therapies associates with the aforementioned conditions. For example, each of the compounds of formulae I and II may be formulated with agents to prevent nausea, hypersensitivity, and gastric irritation, such as antiemetics, and H_1 and H_2 antihistamines. The above therapeutic agents, when employed in combination with the compound of formulae I or II, may be used in those amounts indicated in the Physicians' Desk Reference (PDR) or as otherwise determined by one of ordinary skill in the art.

Furthermore, compounds of formulae I or II may be administered in combination with other anti-cancer and cytotoxic agents and treatments useful in the treatment of cancer or other proliferative diseases. Especially useful are anti-cancer and cytotoxic drug combinations wherein the second drug chosen acts in a different manner or different phase of the cell cycle, e.g., S phase, than the present compounds of formula I and II which exert their effects at the G_2-M phase. Example classes of anti-cancer and cytotoxic agents include, but are not limited to, alkylating agents, such as nitorgen mustards, alkyl sulfonates, nitrosoureas, ethylenimines, and triazenes; antimetabolites, such as folate antagonists, purine analogues, and pyrimidine analogues; antibiotics, such as anthracyclines, bleomycins, mitomycin, dactinomycin, and plicamycin; enzymes, such as L-asparaginase; farnesyl-protein transferase inhibitors; hormonal agents, such as glucocorticoids, estrogens/antiestrogens, androgens/antiandrogens, progestins, and luteinizing hormone-releasing hormone anatagonists, octreotide acetate;

microtubule-disruptor agents, such as ecteinascidins or their analogs and derivatives; microtubule-stabilizing agents such as paclitaxel (Taxol®), docetaxel (Taxotere®); plant-derived products, such as vinca alkaloids, epipodophyllotoxins, taxanes; and topoisomerase inhibitors; prenyl-protein transferase inhibitors; and miscellaneous agents such as, hydroxyurea, procarbazine, mitotane, hexamethylmelamine, platinum coordination complexes such as cisplatin and carboplatin; and other agents used as anti-cancer and cytotoxic agents such as biological response modifiers, growth factors; immune modulators, and monoclonal antibodies. Compounds represented by formulae I and II may also be used in conjunction with radiation therapy.

Representative examples of these classes of anti-cancer and cytotoxic agents include, but are not limited to, mechlorethamine hydrochlordie, cyclophosphamide, chlorambucil, melphalan, ifosfamide, busulfan, carmustin, lomustine, semustine, streptozocin, thiotepa, dacarbazine, methotrexate, thioguanine, mercaptopurine, fludarabine, pentastatin, cladribin, cytarabine, fluorouracil, doxorubicin hydrochloride, daunorubicin, idarubicin, bleomycin sulfate, mitomycin C, actinomycin D, safracins, saframycins, quinocarcins, discodermolides, vincristine, vinblastine, vinorelbine tartrate, etoposide, teniposide, paclitaxel, tamoxifen, estramustine, estramustine phosphate sodium, flutamide, buserelin, leuprolide, pteridines, diyneses, levamisole, aflacon, interferon, interleukins, aldesleukin, filgrastim, sargramostim, rituximab, BCG, tretinoin, irinotecan hydrochloride, betamethosone, gemcitabine hydrochloride, altretamine, and topoteca and any analogs or derivatives thereof.

Preferred members of these classes include, but are not limited to, paclitaxel, cisplatin, carboplatin, doxorubicin, carminomycin, daunorubicin, aminopterin, methotrexate, methopterin, mitomycin C, ecteinascidin 743, porfiromycin, 5-fluorouracil, 6-mercaptopurine, gemcitabine, cytosine arabinoside, podophyllotoxin or podophyllotoxin derivatives such as etoposide, etoposide phosphate or teniposide, melphalan, vinblastine, vincristine, leurosidine, vindesine, and leurosine.

Examples of anti-cancer and other cytotoxic agents include the following: cyclin dependent kinase inhibitors as found in WO 99/24416; and prenyl-protein transferase inhibitors as found in WO 97/30992 and WO 98/54966.

Without being bound by any theory regarding mechanism or morphology, the compounds represented by formulae I and II may also be used to treat conditions other than cancer or other proliferative diseases. Such conditions include, but are not limited to viral infections such as herpesvirus, poxvirus, Epstein-Barr virus, Sindbis virus and adenovirus; autoimmune diseases such as systemic lupus erythematosus, immune mediated glomerulonephritis, rheumatoid arthritis, psoriasis, inflammatory bowel diseases and autoimmune diabetes mellitus; neurodegenerative disorders such as Alzheimer's disease, AIDS-related dementia, Parkinson's disease, amyotrophic lateral sclerosis, retinitis pigmentosa, spinal muscular atrophy and cerebellar degeneration; AIDS; myelodysplastic syndromes; aplastic anemia; ischemic injury associated myocardial infarctions; stroke and reperfusion injury; restenosis; arrhythmia; atherosclerosis; toxin-induced or alcohol induced liver diseases; hematological diseases such as chronic anemia and aplastic anemia; degenerative diseases of the musculoskeletal system such as osteoporosis and arthritis; aspirin-sensitive rhinosinusitis; cystic fibrosis; multiple sclerosis; kidney diseases; and cancer pain.

The effective amount of a compound represented by formulae I and II may be determined by one of ordinary skill in the art, and includes exemplary dosage amounts for a human of from about 0.05 mg/kg/day to about 200 mg/kg/day, which may be administered in a single dose or in the form of individual divided doses, such as from 1 to about 4 times per day. Preferably, the compounds are administered in a dosage of less than about 100 mg/kg/day, in a single dose or in about 2 to about 4 divided doses. It will be understood that the specific dose level and frequency of dosage for any particular subject may be varied and will depend upon a variety of factors including the activity of the specific compound employed, the metabolic stability and length of action of that compound, the species, age, body weight, general health, sex and diet of the subject, the mode and time of administration, rate of excretion, drug combination, and severity of the particular condition. Preferred subjects for treatment include animals, most preferably mammalian species such as humans, and domestic animals such as dogs, cats and the like, subject to the aforementioned disorders.

The compounds represented by formulae I and II, particularly the latter, are disadvantageous from the viewpoint of compounding a suitable formulation for administration in that they possess very low solubility in aqueous media, rapidly degrade in contact with aqueous media, are sensitive to low pH when in solution, are light sensitive, are "Class D" cytotoxic and have exceptionally poor wetting characteristics. Any one or two of these characteristics might be compensated for in compounding a pharmaceutical formulation for intravenous administration, but the combination of all of them presents a formidable challenge to the pharmaceutical compounding chemist. Given the constraint that materials to be utilized in compounding an intravenous formulation must be approved for intravenous administration, the formulation provided in accordance with the present invention unexpectedly was found to be suitable for overcoming the disadvantageous properties of the subject epothilone analogs as noted above. Initially, because of the fact that the subject epothilone analogs are poorly soluble in aqueous media and, in fact, rapidly degrade in contact therewith, it was decided that they should be formulated in lyophilized form.

It has been found that a suitable media to form a solution of the subject compounds for lyophilization is a mixture of tertiary-butanol and water for injection. This mixture must be at least about 50% v/v, preferably from about 50% to about 80% v/v tertiary butanol to prevent degradation of the subject epothilone analogs. Further, due to the exceptionally poor wetting characteristics of the subject epothilone analogs, the initial solution must be effected utilizing a mixture of at least about 60% v/v, preferably from about 60% to about 95% v/v, tertiary butanol and water. Once the solution is made, the requisite amount of water or tertiary-butanol-water mixture can be added to achieve the final concentration for lyophilization as stated above.

It has unexpectedly been found that the stability of the subject epothilone analogs can be significantly enhanced by carrying out the preparation of the solution at a temperature below ambient, preferably from about 5° C. to about 15° C., more preferably about 5° C. Further, both the process of forming the solution and subsequent lyophilization are to be carried out in vessels such that the epothilone analogs are protected from exposure to light. It is also beneficial to carry out the lyophilization in comparatively small batches so that the epothilone analogs are exposed to an aqueous medium for a minimum amount of time.

The primary drying stage of lyophilization of the solution formed as described above is carried out at temperatures

from about −10° C. to about −40° C., preferably about −25° C., under high vacuum, i.e., from about 50 millitorr to about 300 millitorr, preferably about 200 millitorr, for an extended period, i.e., from about 24 hours to about 96 hours, preferably about 48 hours. Lyophilization in this temperature range produces an amorphous product which is desirable for an intravenous preparation. Those of ordinary skill in the art will appreciate that conventional procedures, such as powder X-ray diffraction, can be utilized to confirm the amorphous nature of the lyophilized product.

The residual solvents in the product are removed by a secondary drying stage that is carried out at comparatively low temperatures, i.e., from about 10° C. to about 30° C., preferably about 25° C., under high vacuum, i.e., from about 50 millitorr to about 300 millitorr, preferably about 150 millitorr for an extended period, i.e., from about 24 hours to about 96 hours, preferably about 48 hours.

It has unexpectedly been found that the stability of lyophilized epothilone analogs described herein are not enhanced by excipients commonly utilized for such purposes, such as lactose, mannitol, dextran and the like. Certain of these excipients may actually have a negative effect on the stability of the lyophilized product (lyophile). Hence, the epothilone analogs formulated in accordance with the present invention are lyophilized neat, i.e., without any excipient.

The lyophilized epothilone analogs represented by formulae I and II are reconstituted with a mixture of equal parts by volume of Dehydrated Alcohol, USP and a nonionic surfactant, preferably a polyoxyethylated castor oil surfactant available from GAF Corporation, Mount Olive, N.J., under the trademark, Cremophor EL. The lyophilized product and vehicle for reconstitution are packaged separately in appropriately light-protected vials. To minimize the amount of surfactant in the reconstituted solution, only a sufficient amount of the vehicle is provided to form a solution having a concentration of about 2 mg/mL to about 4 mg/mL of the epothilone analog. Once dissolution of the drug is achieved, the resulting solution is further diluted prior to injection with a suitable parenteral diluent. Such diluents are well known to those of ordinary skill in the art. These diluents are generally available in clinical facilities. It is, however, within the scope of the present invention to package the subject epothilone analogs with a third vial containing sufficient parenteral diluent to prepare the final concentration for administration. A preferred diluent is Lactated Ringer's Injection. The final concentration for administration would preferably contain from about 0.1 mg/mL to about 0.9 mg/mL of the epothilone analog.

The final dilution of the reconstituted epothilone analog in the formulation of the invention may be carried out with other preparations having similar utility, for example, 5% Dextrose Injection, Lactated Ringer's and Dextrose Injection, Sterile Water for Injection, and the like. However, because of its narrow pH range, pH 6.0 to 7.5, Lactated Ringer's Injection is preferred. Per 100 mL, Lactated Ringer's Injection contains Sodium Chloride USP 0.6 g, Sodium Lactate 0.31 g, Potassium chloride USP 0.03 g and Calcium Chloride.$2H_2O$ USP 0.02 g. The osmolarity is 275 mOsmol/L, which is very close to isotonicity.

The constituted preparation according to the present invention, i.e., the solution of the epothilone analog in the alcohol-surfactant vehicle, can be stored for up to about 24 hours before being further diluted for administration. It has been found that the incidence of allergic reactions encountered due to the presence of the surfactant in the formulation is minimized by keeping its concentration at the minimum necessary to effect solution of the epothilone analog. Further, the incidence of such reactions is about the same as has been experienced with other parenterally administered pharmaceuticals containing it, such as cyclosporine. This observed level of allergic reaction with the present formulation is significantly lower that has been experienced with certain other oncology agents, such as Paclitaxel. The final dilution is administered by intravenous infusion, typically over a period of up to an hour.

The following non-limiting example serves to illustrate the practice of the present invention.

EXAMPLE

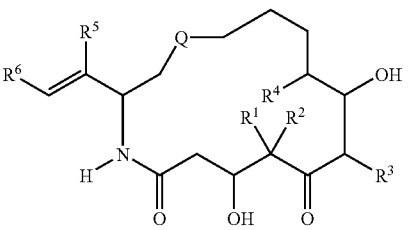
[1S-[1R*,3R*(E),7R*,10S*,11R*,12R*,16S*]]-7,11-Dihydroxy-8,8,10,12,16-pentamethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-4-aza-17-oxabicyclo[14.1.0]heptadecane-5,9-dione, 9.86 g, was wetted/partially dissolved with 600 mL of a 9:1 mixture of tertiary butanol and Water for Injection USP which had been pre-cooled to 5° C. Once the drug powder had become completely wetted, dissolution was completed by the addition of 600 mL of a 1:9 mixture of tertiary butanol and Water for Injection and 766 mL of a 1:1 mixture of tertiary butanol and Water for Injection which likewise had been pre-cooled to 5° C. thereby making the final solution a 1:1 mixture. The dissolution was carried out under protection from light.

The solution formed above was promptly lyophilized in a Virtis INOTOP lyophilizer at −16° C. under light protectant conditions over a period of 48 hours. The resultant lyophilized product (lyophile) was then further dried at 15° C. under high vacuum for 48 hours. No detectable degradation of the drug was observed during these procedures. The lyophile was packaged under sterile conditions into 30 mL vials, each containing 10 mg of drug and standard excess to allow for vial/needle/syringe loss.

The lyophile is reconstituted with 5.5 mL of a 1:1 volume mixture of Dehydrated Alcohol USP and Cremophor EL®, which typically will be supplied with the drug in a separate vial, to achieve a final drug concentration of 2 mg/mL. Once dissolution is effected by gently swirling the vial, the resultant solution is diluted to achieve a concentration of 0.2 mg/mL by the addition of 9 mL of Lactated Ringer's for Injection for each milliliter of constituted drug product.

What is claimed is:

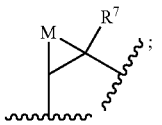
1. A process for formulating an epothilone analog represented by formula I:



I

and/or a pharmaceutically-acceptable salt, geometric, optical, or stereoisomer thereof, wherein:

Q is



M is oxygen;

each R^1, R^2, R^3, R^4, R^5, and R^7 is, independently, selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, substituted aryl and heterocyclo, and wherein R^1 and R^2 are alkyl, they can be joined to form cycloalkyl;

R^6 is selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, cycloalkyl, and heterocyclo;

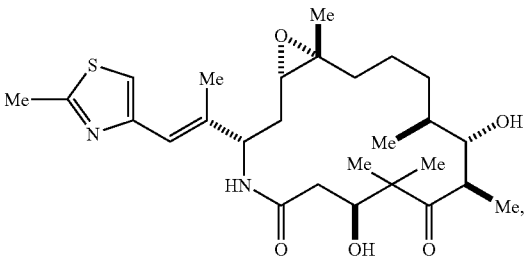
and any salts, solvates, or hydrates thereof, comprising the following steps:

a) dissolving said epothilone analog in a mixture of at least about 50% by volume tertiary-butanol in water to form a solution;

b) performing primary drying of said solution at a temperature of from about −10° C. to about −40° C. under vacuum of from about 50 millitorr to about 300 millitorr for from about 24 hours to about 96 hours to form a primary lyophilized product; and

c) performing secondary drying of the primary lyophilized product at a temperature of from about 10° C. to about 30° C. under vacuum of from about 50 millitorr to about 300 millitorr for from about 24 hours to about 96 hours to provide a lyophilized product of the epothilone analog.

2. The process of claim **1** wherein said epothilone analog is represented by formula II:



II

and/or a pharmaceutically-acceptable salt, geometric, optical, or stereoisomer thereof.

3. The process of claim **1** wherein step a) comprises first, wetting said epothilone analog with a mixture of at least about 60% tertiary-butanol in water, and then adding sufficient water, or a mixture of tertiary-butanol and water, so that the resulting solution contains from about 2 mg/mL to about 30 mg/mL of said epothilone analog in a mixture of from about 50% to about 80% by volume tertiary-butanol in water.

4. The process of claim **2** wherein step a) comprises first, wetting said epothilone analog with a mixture of at least about 60% tertiary-butanol in water, and then adding sufficient water, or a mixture of tertiary-butanol and water, so that the resulting solution contains from about 2 mg/mL to about 30 mg/mL of said epothilone analog in a mixture of from about 50% to about 80% by volume tertiary-butanol in water.

5. The process of claim **3** wherein in step a) said epothilone analog is initially wetted with a mixture of from about 60% to about 95% by volume tertiary-butanol in water.

6. The process of claim **4** wherein in step a) said epothilone analog is initially wetted with a mixture of from about 60% to about 95% by volume tertiary-butanol in water.

7. The process of claim **2** wherein said primary drying in step b) is carried out at a temperature of from about −25° C. to −40° C. and a pressure of from about 200 to 300 millitorr.

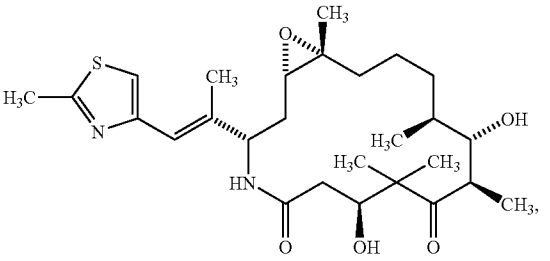
8. The process of claim **2** wherein said secondary drying in step c) is carried out at a temperature of from about 25° C. to 30° C. and a pressure of from about 150 to 300 millitorr.

9. The process of claim **1**, further comprising the step of:

d) packaging said lyophilized product of the epothilone analog in a first vial and packaging in a second vial a sufficient quantity of a mixture comprising at least one suitable nonionic surfactant and at least one dehydrated alcohol to effect reconstitution of the lyophilized product.

10. The process of claim **9** wherein the mixture comprises about equal parts by volume of anhydrous ethanol and the at least one nonionic surfactant.

11. The process of claim **9** wherein said epothilone analog is:



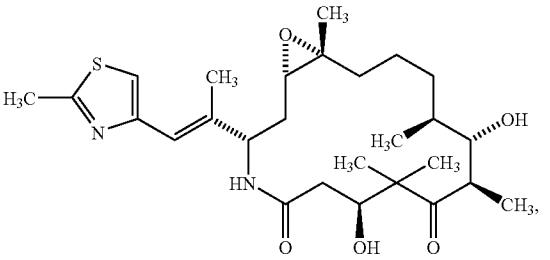
and/or a pharmaceutically-acceptable salt, geometric, optical, or stereoisomer thereof.

12. The process of claim **9** wherein said surfactant is polyethoxylated castor oil.

13. The process of claim **11** wherein said surfactant is polyethoxylated castor oil.

14. The process of claim **9** wherein said second vial contains an amount of said mixture sufficient to form a solution of from about 2 mg/mL to about 4 mg/mL of said epothilone analog therein.

15. A process for formulating an epothilone analog having the formula:



and/or a pharmaceutically-acceptable salt, geometric, optical, or stereoisomer thereof, comprising:

a) dissolving said epothilone analog in a mixture of tertiary butanol and water to form a solution, wherein the mixture comprises at least about 50% by volume tertiary butanol;

b) performing primary drying of said solution at a temperature, chamber pressure and period of time sufficient to form a primary lyophilized product; and

c) performing secondary drying of the primary lyophilized product at a temperature, chamber pressure and for a period of time sufficient to form a lyophilized product of the epothilone analog.

16. The process of claim **15**, wherein said step a) of dissolving said epothilone analog is carried out at a temperature below ambient temperature.

17. The process of claim **15**, wherein said step a) of dissolving said epothilone analog comprises first, wetting said epothilone analog with a mixture of at least about 60% by volume tertiary-butanol in water, and then adding sufficient water, or a mixture of tertiary-butanol and water, so that the resulting solution contains at least about 50% by volume tertiary-butanol in water.

18. The process of claim **15**, wherein said step a) of dissolving said epothilone analog is carried out in the absence of an excipient.

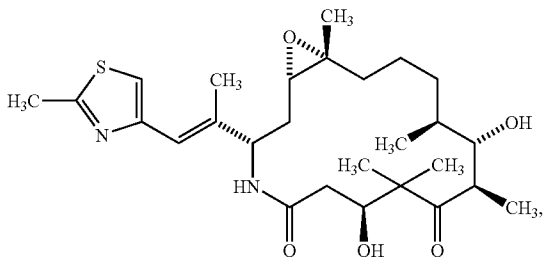
19. The process of claim **15** wherein said primary drying in step b) is carried out at a temperature of from about −10° C. to −40° C. and at a chamber pressure of from about 50 to 300 millitorr for a period of up to about 96 hours.

20. The process of claim **19** wherein said secondary drying in step c) is carried out at a temperature of from about 10° C. to 300° C. and at a chamber pressure of from about 150 to 300 millitorr for a period of up to about 96 hours.

21. The process of claim **15**, further comprising the step of:

d) packaging said lyophilized product of the epothilone analog in a first vial and packaging in a second vial a sufficient quantity of a solvent mixture to effect reconstitution of the epothilone analog, wherein the solvent mixture of the second vial comprises at least one suitable nonionic surfactant and at least one anhydrous alcohol.

22. A process for formulating an epothilone analog represented by formula II:



and/or a pharmaceutically-acceptable salt, geometric, optical, or stereoisomer thereof, comprising:

a) dissolving said epothilone analog to form a solution, comprising first, wetting said epothilone analog with a mixture of at least about 60% by volume tertiary-butanol in water, and then adding sufficient water, or a mixture of tertiary-butanol and water, so that the resulting solution contains at least about 50% by volume tertiary-butanol in water, wherein said step of dissolving is carried out at a temperature below ambient temperature;

b) performing primary drying of said solution at a temperature, chamber pressure and for a period of time sufficient to form a primary lyophilized product; and

c) performing secondary drying of the primary lyophilized product at temperature, chamber pressure and for a period of time sufficient to form a lyophilized product of the epothilone analog.

23. The process of claim **22** wherein,

step b) of primary drying of said solution is performed at a temperature of about −10° C. to about −40° C. under vacuum of from about 50 millitorr to about 300 millitorr for from about 24 hours to about 96 hours; and

step c) of secondary drying is performed at a temperature of from about 10° C. to about 30° under vacuum of from about 50 millitorr to about 300 millitorr for from about 24 hours to about 96 hours to provide a lyophilized product of the epothilone analog.

24. The process of claim **23** wherein,

step a) of dissolving said epothilone analog is carried out at a temperature of from about −5° C. to about 15° C.

25. The process of claim **24** wherein,

step a) of dissolving said epothilone analog is carried out in the absence of an excipient.

26. The process of claim **24**, further comprising the step of:

d) packaging said lyophilized product of the epothilone analog in a first vial and packaging in a second vial a sufficient quantity of a solution to effect reconstitution of the lyophilized epothilone analog, wherein the solution of the second vial comprises about equal amounts of at least one suitable nonionic surfactant and at least one anhydrous alcohol.

27. A pharmaceutical preparation comprising a lyophilized epothilone analog prepared according to claim **1**.

28. A pharmaceutical preparation comprising a lyophilized epothilone analog prepared according to claim **2**.

29. A pharmaceutical preparation comprising a lyophilized epothilone analog prepared according to claim **15**.

30. A pharmaceutical preparation comprising a lyophilized epothilone analog prepared according to claim **20**.

31. A pharmaceutical preparation comprising a lyophilized epothilone analog prepared according to claim **24**.

32. A pharmaceutical product comprising at least a first and a second vial wherein the first vial contains a lyophilized epothilone analog prepared according to claim **2**, and the second vial contains a sufficient quantity of a solution to effect reconstitution of the lyophilized epothilone analog, wherein the solution of the second vial comprises about equal amounts of at least one suitable nonionic surfactant and at least one anhydrous alcohol.

33. A method of treating a patient comprising, mixing the contents of the first and second vials of the pharmaceutical product of claim **32** to provide an epothilone analog solution, diluting the epothilone analog solution with a quantitiy of a suitable parenteral diluent to prepare an intravenous formulation, and administering the intravenous formulation to the patient.

34. A pharmaceutical product comprising at least a first and a second vial wherein the first vial contains a lyophilized epothilone analog prepared according to claim **15**, and the second vial contains a sufficient quantity of a solution to effect reconstitution of the lyophilized epothilone analog,

wherein the solution of the second vial comprises about equal amounts of at least one suitable nonionic surfactant and at least one anhydrous alcohol.

35. A method of treating a patient comprising, mixing the contents of the first and second vials of the pharmaceutical product of claim **34** to provide an epothilone solution, diluting the epothilone solution with a quantitiy of a suitable parenteral diluent to prepare an intravenous formulation, and administering the intravenous formulation to the patient.

36. The process of claim **15**, wherein said step a) of dissolving said epothilone analog is carried out at a temperature in the range of from about 5° C. to about 15° C.

* * * * *

Exhibit C

US00RE41393E

(19) **United States**

(12) **Reissued Patent**

Lee

(10) **Patent Number: US RE41,393 E**

(45) **Date of Reissued Patent: Jun. 22, 2010**

(54) **TREATMENT OF REFRACTORY TUMORS USING EPOTHILONE DERIVATIVES**

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(73) Assignee: **Bristol-Myers Squibb Company**, Princeton, NJ (US)

(21) Appl. No.: **11/346,579**

(22) Filed: **Feb. 2, 2006**

Related U.S. Patent Documents

Reissue of:

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Issued: **Feb. 3, 2004**
Appl. No.: **10/072,123**
Filed: **Feb. 8, 2002**

U.S. Applications:

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(51) **Int. Cl.**
A61K 31/425 (2006.01)

(52) **U.S. Cl.** **514/365**; 514/183; 514/922

(58) **Field of Classification Search** 514/183, 514/186, 365, 922
See application file for complete search history.

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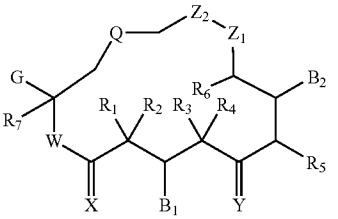
(57) **ABSTRACT**

Methods of treating tumors in a mammal, especially a human that has demonstrated resistance to other chemotherapeutic agents, is disclosed. Specifically, methods of the present invention are effective in tumors that have initially been unresponsive to taxane therapy, or have developed resistance during the course of treatment. The methods of the present invention comprise administering epothilone derivatives selected from those represented by the formula:

I



The subject epothilone derivatives are advantageous in addition to their enhanced potency and effectiveness against tumors that have demonstrated resistance to therapy with taxane oncology agents in that they are efficacious upon oral administration.

13 Claims, 3 Drawing Sheets

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BMS-247550	
Cell Line	IC_{50} (μM)
A2780/DDP-S	0.0028
A2780/DDP-R	0.0018
A2780/TAX-S	0.0026
A2780/TAX-R	0.0049
OVCAR-3	0.0018
MCF-7	0.0027
SKBR3	0.0023
LNCAP	0.0015
PC3	0.0046
HCT116	0.0026
HCT116/VM46	0.0245
HCT116/VP35	0.0020
LS174T	0.0058
MIP	0.0248
CACO-2	ND
A549	0.0052
LX-1	0.0031
A431	0.0014
CCRF-CEM	0.0060
HL60	ND
K562	0.0029
ABAE	ND
HS27	ND
M109	0.0029
MLF	0.0345
Median IC50	0.0029
GeoMean IC50	0.0039
Min IC50	0.0014
Max IC50	0.0345
Range(LogIC50)	1.41

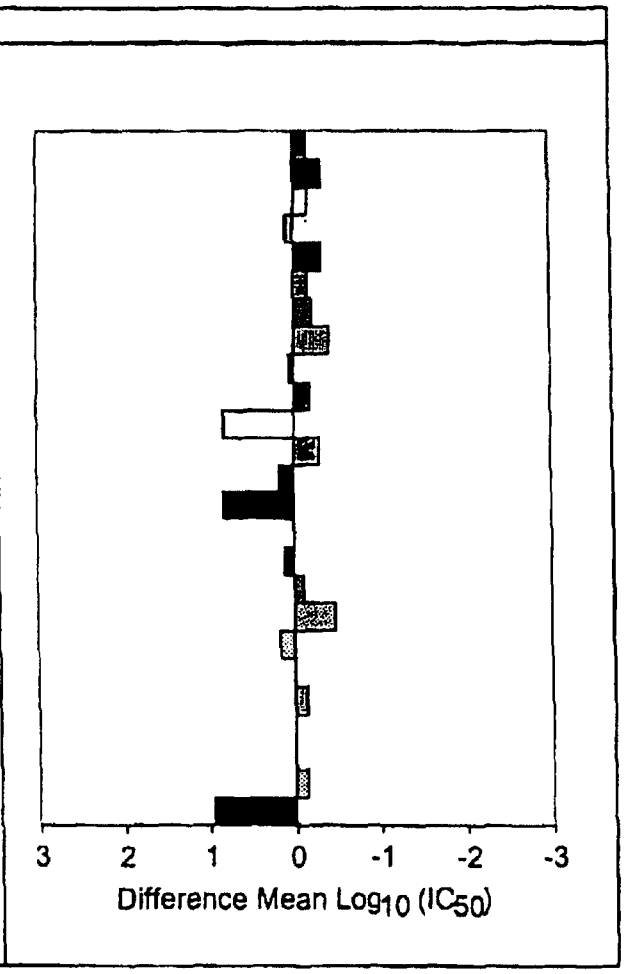


Figure 1. **Cytotoxicity spectrum of BMS-247550 versus a panel of tumor cell lines. The mean bar graph, on the right, graphically depicts the difference between the log of the individual cell line IC_{50} values relative to the mean log of all the IC_{50} values. Right projecting bars indicate sensitive cells and left projecting bars indicate resistant cell lines. Mean IC_{50} = 3.9 nM. ND= Not done.**

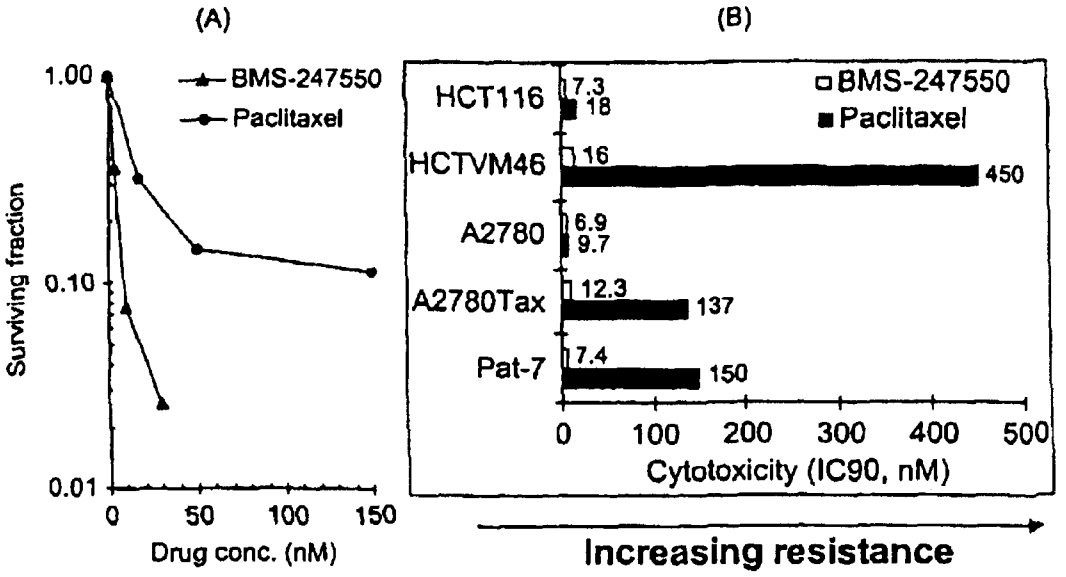


Figure 2. **BMS-247550 retains its anticancer cytotoxicity against tumor types that had developed resistance to paclitaxel. (A) Clonogenic cell survival of Pat-7 ovarian carcinoma cells following a 16 hr exposure to BMS-247550 or paclitaxel. (B) Comparative cytotoxic potency (IC_{90}) of BMS-247550 and paclitaxel in five human tumor lines: HCT116 human colon carcinoma; HCT116/VM46 (MDR-resistant variant derived from HCT116); A2780 human ovarian ca.; A2780Tax (paclitaxel-resistant variant due to a mutation in beta-tubulin; Pat-7 human ovarian ca. (derived from a patient who had developed resistance to Taxol® monotherapy). IC_{90}'s are the concentration of the agent required to reduce colony formation by 90%. IC_{90} values are shown next to the bar graphs.**

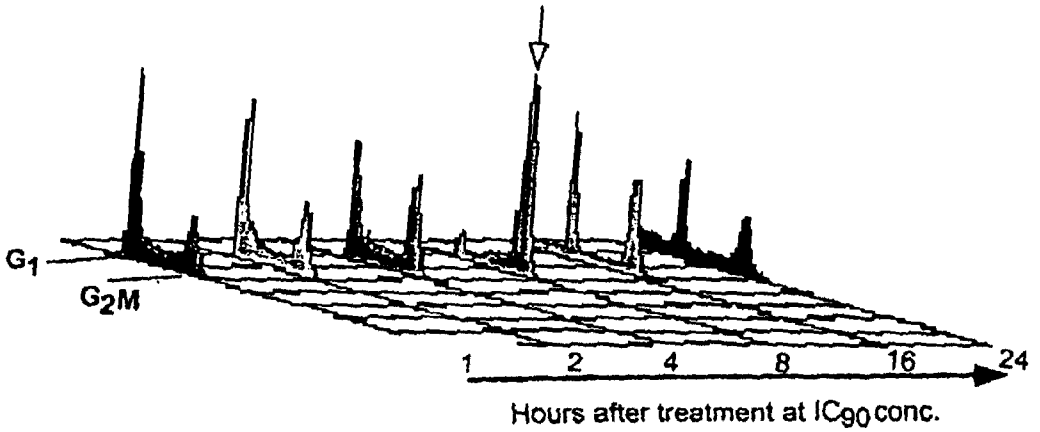


Figure 3. Time-course of the mitotic blockade induced by the incubation of HCT116 colon carcinoma cells in cell culture media containing 7.5 nM (IC_{90}) BMS-247550. The arrow indicates the position of the G_2M cell population.

TREATMENT OF REFRACTORY TUMORS USING EPOTHILONE DERIVATIVES

Matter enclosed in heavy brackets [ ] appears in the original patent but forms no part of this reissue specification; matter printed in italics indicates the additions made by reissue.

CROSS-REFERENCE TO RELATED APPLICATIONS

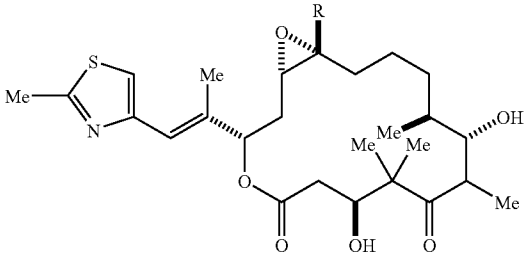
This application claims priority from provisional application serial No. 60/269,836, filed Feb. 20, 2001, incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

The present invention relates to the use of certain potent epothilone analogs in the treatment of tumors that have demonstrated resistance to therapy with other chemotherapeutic agents.

BACKGROUND OF THE INVENTION

Epothiolones are macrolide compounds that find utility in the pharmaceutical field. For example, epothilones A and B having the structures:



Epothilone A R = H

Epothilone B R = Me

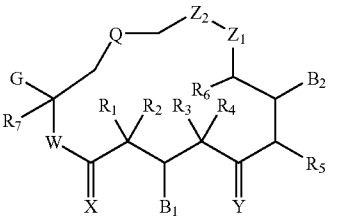
may be found to exert microtubule-stabilizing effects similar to paclitaxel (TAXOL®) and hence cytotoxic activity against rapidly proliferating cells, such as tumor cells or other hyperproliferative cellular diseases. See, Hofle et al., Angew. Chem. Int. Ed. Engl., Vol. 35, No. 13/14, 1567–1569 (1996); WO93/10121 published May 27, 1993; and WO97/19086 published May 29, 1997.

Derivatives and analogs of epothilones A and B have been synthesized and may be used to treat a variety of cancers and other abnormal proliferative diseases. Such analogs are disclosed in Hofle et al., Id.; Nicolaou et al., Agnew. Chem. Int. Ed. Eng., Vol.36, No. 19, 2097–2103 (1997); and Su et al., Agnew. Chem. Int. Ed. Engl., Vol. 36, No. 19, 2093–2097 (1997). In some instances, epothilone derivatives have demonstrated enhanced properties over epothilones A and B. The present invention is concerned with the discovery that certain epothilone derivatives may be utilized to treat cancers that have demonstrated resistance to other chemotherapeutic agents, such as oncolytic agents of the taxane family of compounds.

SUMMARY OF THE INVENTION

In accordance with the present invention, tumors demonstrating a clinical resistance to treatment with other chemotherapeutic agents, such as taxane oncolytic agents, may be treated with an epothilone derivative selected from those represented by formula I:

I



wherein B_1, B_2, G, Q, X, Y, Z_1, Z_2, and R_1 through R_7 have the meanings given below. The compounds represented by formula I have previously demonstrated significantly enhanced potency over known chemotherapeutic agents, for example, epothilones A and B above and certain others including those in the taxane series. Compounds represented by formula I are further advantageous in that, unlike most oncology agents, they are efficacious via oral administration.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** is a bar graph showing the cytotoxicity spectrum of a compound of the invention against a panel of tumor cell lines.

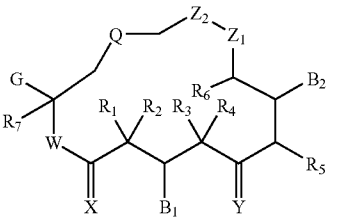
FIG. **2** is a bar graph showing the cytotoxicity of a compound of the invention against paclitaxel-resistant tumors.

FIG. **3** shows the mitotic blockade induced by a compound of the invention.

DETAILED DESCRIPTION OF THE INVENTION

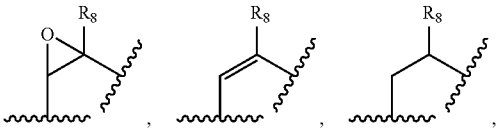
Processes of the present invention provide advantageous treatment for tumors that have demonstrated resistance to treatment with chemotherapeutic agents, such as those of the taxane family. The term "resistance to treatment" as utilized herein includes both tumors that are initially unresponsive to treatment with a chemotherapeutic agent as well as tumors that are initially responsive, but develop resistance over the course of treatment. Compounds useful in the subject method are epothilones, a class of oncology chemotherapeutic agents chemically distinct from the taxane family of oncology agents. The subject epothilone derivatives are represented by formula I:

I



wherein

Q is selected from the group consisting of



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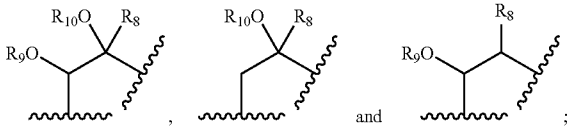
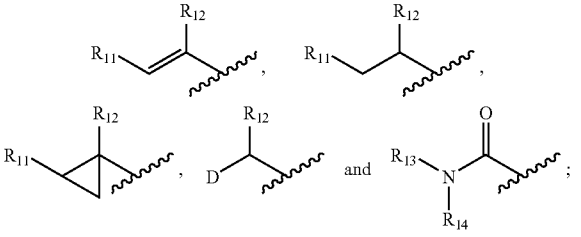


G is selected from the group consisting of alkyl, substituted alkyl, aryl, substituted aryl, heterocyclo,



W is O or N R_{15};

X is O or H, H;

Y is selected from the group consisting of O; H, OR_{16}; OR_{17}, OR_{17}; NOR_{18}; H, $NHOR_{19}$; H, $NR_{20}R_{21}$; H, H; and CHR_{25}; wherein OR_{17}, OR_{17} can be cyclic ketal;

each Z_1 and Z_2 is, independently, selected from the group consisting of CH_2, O, NR_{23}, S, and SO_2, wherein only one of Z_1 and Z_2 can be a heteroatom;

each B_1 and B_2 is, independently, selected from the group consisting of OR_{24}, $OCOR_{25}$, and O—C(═O)—$NR_{26}R_{27}$, and when B_1 is H and Y is OH, H, they can form a six-membered ring ketal or acetal;

D is selected from the group consisting of $NR_{28}R_{29}$, $NR_{30}COR_{31}$ and saturated heterocycle;

each R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_{13}, R_{14}, R_{18}, R_{19}, R_{20}, R_{21}, R_{22}, R_{26} and R_{27} is, independently, selected from the group consisting of H, alkyl, substituted alkyl, and aryl, and when R_1 and R_2 are alkyl they can be joined to form cycloalkyl, and when R_3 and R_4 are alkyl they can be joined to form cycloalkyl;

each R_9, R_{10}, R_{16}, R_{17}, R_{24}, R_{25} and R_{31} is, independently, selected from the group consisting of H, alkyl, and substituted alkyl;

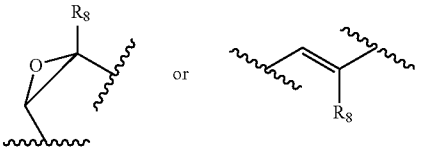
each R_8, R_{11}, R_{12}, R_{28}, R_{30}, R_{32}, and R_{33} is, independently, selected from the group consisting of H, alkyl, substituted alkyl, aryl, substituted aryl, cycloalkyl and heterocyclo; and

each R_{15}, R_{23} and R_{29} is, independently, selected from the group consisting of H, alkyl, substituted alkyl, aryl, substituted aryl, cycloalkyl, heterocyclo, $R_{32}C$═O, $R_{33}SO_2$, hydroxy, O-alkyl or O-substituted alkyl;

and pharmaceutically acceptable salts thereof and any hydrates, solvates or geometric, optical and stereoisomers thereof,

with the proviso that compounds wherein W and X are both O; R_1, R_2 and R_7 are H; R_3, R_4 and R_6 are methyl; R_8 is H or methyl; Z_1 and Z_2 are CH_2; G is 1-methyl-2-(substituted-4-thiazolyl)ethenyl; and Q is as defined above, are excluded.

Preferred compounds in accordance with the present invention are those represented by formula I above wherein Q is



X is O; Y is O; Z_1 and Z_2 are CH_2; and W is NR_{15}.

Another preferred group of compounds in accordance with the present invention is represented below:

[1S-[1R*,3R*(E),7R*,10S*,11R*,12R*,16S*]]-7,11-dihydroxy-8,8,10,12,16-pentamethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-4,13,17-trioxabicyclo[14.1.0]heptadecane-5,9-dione;

[1 S-[1R*,3R*(E),7R*,10S*,11R*,12R*,16S*]]-7,11-dihydroxy-8,8,10,12-tetramethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-4,13,17-trioxabicyclo[14.1.0]heptadecane-5,9-dione;

[4S-[4R*,7S*,8R*,9R*,15R*(E)]]-4,8-dihydroxy-5,5,7,9,13-pentamethyl-16-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-1,10-dioxa-13-cyclohexadecene-2,6-dione;

[4S-[4R*,7S *,8R*,9R*,15R*(E)]]-4,8-dihydroxy-5,5,7,9-tetramethyl-16-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-1,10-dioxa-13-cyclohexadecene-2,6-dione;

[1S-[1R*,3R*(E),7R*,10S*,11R*,12R*,16S*]]-7,11-dihydroxy-8,8,10,12,16-pentamethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-4,14,17-trioxabicyclo[14.1.0]heptadecane-5,9-dione;

[1S-[1R*,3R*(E),7R*,10S*,11R*,12R*,16S*]]-7,11-dihydroxy-8,8,10,12-tetramethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-4,14,17-trioxabicyclo[14.1.0]heptadecane-5,9-dione;

[4S-[4R*,7S*,8R*,9R*,15R*(E)]]-4,8-dihydroxy-5,5,7,9,13-pentamethyl-16-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-1,11-dioxa-13-cyclohexadecene-2,6-dione;

[4S-[4R*,7S*,8R*,9R*,15R*(E)]]-4,8-dihydroxy-5,5,7,9-tetramethyl-16-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-1,11-dioxa-13-cyclohexadecene-2,6-dione;

[1S-[1R*,3R*(E),7R*,10S*,11R*,12R*,16S*]]-7,11-dihydroxy-8,8,10,12,16-pentamethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-4,17-dioxabicyclo[14.1.0]heptadecane-9-one;

[1S-[1R*,3R*(E),7R*,10S*,11R*,12R*,16S*]]-7,11-dihydroxy-8,8,10,12-tetramethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-4,17-dioxabicyclo[14.1.0]heptadecane-9-one; hexamethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-4, 17-dioxabicyclo[14.1.0]heptadecane-5,9-dione;

[1S-[1R*,3R*(E),7R*,10S*,11R*,12R*,16S*]]-7,11-dihydroxy-3,8,8,10,12-pentamethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-4,17-dioxabicyclo[14.1.0]heptadecane-5,9-dione;

[4S-[4R*,7S*,8R*,9R*,15R*(E)]]-4,8-dihydroxy-5,5,7,9,13,16-hexamethyl-16-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-1-oxa-13-cyclohexadecene-2,6-dione;

[4S-[4R*,7S*,8R*,9R*,15R*(E)]]-4,8-dihydroxy-5,5,7,9,16-pentamethyl-16-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-1-oxa-13-cyclohexadecene-2,6-dione;

[1 S-[R*,3R*(E),7R*,10S*,11R*,12R*,16S*]]-7,11-dihydroxy-8,8,10,12,16-pentamethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-4,17-dioxabicyclo[14.1.0]heptadecane-5,9-dione;

[1S-[1R*,3R*(E),7R*,10S*,11 R*,12R*,16S*]]-7,11-dihydroxy-6,8,8,10,12-pentamethyl-3-[1-methyl-2-(2-

methyl-4-thiazolyl)ethenyl]-4,17-dioxabicyclo[14.1.0] heptadecane-5,9-dione;

[1S-[1R*,3R*(E),7R*,10S*,11R*,12R*,16S*]]-7,11-dihydroxy-8,8,10,12,16-pentamethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-4-aza-17-oxabicyclo[14.1.0] heptadecane-5,9-dione;

[1 S-[1R*,3R*(E),7R*,10S*,11R*,12R*,16S*]]-7,11-dihydroxy-8,8,10,12-tetramethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-4-aza-17-oxabicyclo[14.1.0] heptadecane-5,9-dione;

[4S-[4R*,7S *,8R*,9R*,15R*(E)]]-4,8-dihydroxy-5,5,7,9,13-pentamethyl-16-[1-methyl-2-(2-methyl-4-thiazolyl) ethenyl]-1-aza-13-cyclohexadecene-2,6-dione;

[4S-[4R*,7S*,8R*,9R*,15R*(E)]]-4,8-dihydroxy-5,5,7,9-tetramethyl-16-[1-methyl-2-(2-methyl-4-thiazolyl) ethenyl]-1-aza-13-cyclohexadecene-2,6-dione;

[1S-[1R*,3R*(E),7R*,10S*,11R*,12R*,16S*]]-7,11-dihydroxy-4,8,8,10,12,16-hexamethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-4-aza-17-oxabicyclo[14.1.0] heptadecane-5,9-dione;

[1S-[R*,3R*(E),7R*,10S*,11R*,12R*,16S*]]-7,11-dihydroxy-4,8,8,10,12-pentamethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-4-aza-17-oxabicyclo[14.1.0] heptadecane-5,9-dione;

[4S-[4R*,7S*,8R*,9 R*,15R*(E)4,8-dihydroxy-1,5,5,7,9,13-hexamethyl-16-[1-methyl-2-(2-methyl-4-thiazolyl) ethenyl]-1-aza-13-cyclohexadecene-2,6-dione;

[4S-[4R*,7S*,8R*,9R*,15R*(E)]]-4,8-dihydroxy-1,5,5,7,9-pentamethyl-16-[1-methyl-2-(2-methyl-4-thiazolyl) ethenyl]-1-aza-13-cyclohexadecene-2,6-dione;

[1S-[1R*,3R*(E),7R*,10S*,11R*,12R*,16S*]]-7,11-dihdyroxy-8,8,10,12,16-pentamethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-13-aza-4,17-dioxabicyclo [14.1.0]heptadecane-5,9-dione;

[1 S-[1R*,3R*(E),7R*,10S*,11R*,12R*,16S*]]-7,11-dihydroxy-8,8,10,12-tetramethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-13-aza-4,17dioxabicyclo [14.1.0]heptadecane-5,9-dione;

[4S-[4R*,7S*,8R*,9R*,15R*(E)]]-4,8-dihydroxy-5,5,7,9,13-pentamethyl-16-[1-methyl-2-(2-methyl-4-thiazolyl) ethenyl]-10-aza-1-oxa-13-cyclohexadecene-2,6-dione;

[4S-[4R*,7S*,8R*,9R*,15R*(E)]]-4,8-dihydroxy-5,5,7,9-tetramethyl-16-[1-methyl-2-(2-methyl-4-thiazolyl) ethenyl]-10-aza-1-oxa-13-cyclohexadecene-2,6-dione;

[1S-[1R*,3R*(E),7R*,10S*,11R*,12R*,16S*]]-7,11-dihydroxy-8,8,10,12,16-pentamethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-14-aza-4,17-dioxabicyclo [14.1.0]heptadecane-5,9-dione;

[1S-[R*,3R*(E),7R*,10S*,11R*,12R*,16S*]]-7,11-dihydroxy-8,8,10,12-tetramethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-14-aza-4,17-dioxabicyclo [14.1.0]heptadecane-5,9-dione;

[4S-[4R*,7S,8R*,9R*,15R*(E)]]-4,8-dihydroxy-5,5,7,9,13-pentamethyl-16-[1-methyl-2-(2-methyl-4-triazolyl) ethenyl]-11-aza-1-oxa-13-cyclohexadecene-2,6-dione;

[4S-[4R*,7S*,8R*,9R*15R*(E)]]-4,8-dihydroxy-5,5,7,9-tetramethyl-16-[1-methyl-2-(2-methyl-4-thiazolyl) ethenyl]-11-aza-1-oxa-13-cyclohexadecene-2,6-dione;

[1 S-[1R*,3R*,7R*,10S*,11R*,12R*,16S*]]-N-phenyl-7,11-dihydroxy-8,8,10,12,16-pentamethyl-5,9-dioxo-4,17-dioxabicyclo[14.1.0]heptadecane-3-carboxamide;

[1S-[1R*,3R*,7R*,10S*,11R*,12R*,16S*]]-N-phenyl-7,11-dihydroxy-8,8,10,12-tetramethyl-5,9-dioxo-4,17-dioxabicyclo[14.1.0]heptadecane-3-carboxamide;

4S-[4R*,7S *,8R*,9R*,15R*]]-N-phenyl-4,8-dihydroxy-5,5,7,9,13-pentamethyl-2,6-dioxo-1-oxa-13-cyclohexadecene-16-carboxamide;

[4S-[4R*,7S,8R*,9R*,15R*]]-N-phenyl-4,8-dihydroxy-5,5,7,9-tetramethyl-2,6-dioxo-1-oxa-13-cyclohexadecene-16-carboxamide;

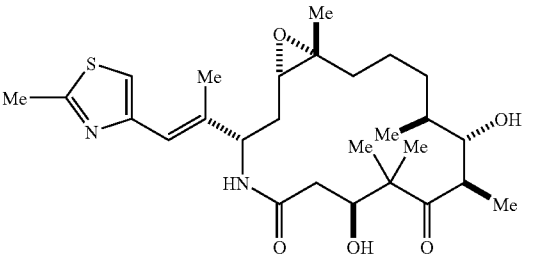
[1S-1R*,3R*(E),7R*,10S*,11R*,12R*,16S*]]-7,11-dihydroxy-8,8,10,12,16-pentamethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)cyclopropyl]-4,17-dioxabicyclo [14.1.0]heptadecane-5,9-dione;

1[S-[1R*,3R*(E),7R*,10S*,11R, 12R*,16S*]]-7,11-dihydroxy-8,8,10,12-tetramethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)cyclopropyl]-4,17-dioxabicyclo [14.1.0]heptadecane-5,9-dione; and

[4S-[4R*,7S*,8R*,9R*,15R*(E)]]-4,8-dihydroxy-5,5,7,9,13-pentamethyl-16-[1-methyl-2-(2-hydroxymethyl-4-thiazolyl)ethenyl]-1-aza-13(Z)-cyclohexadecene-2,6-dione;

and pharmaceutically acceptable salts, solvates and hydrates thereof.

A particular preferred compound in accordance with the present invention is represented by the formula:



This compound chemically is [1S-[1R*,3R*(E),7R*,10S*, 11R*,12R*,16S*]]-7,11-dihydroxy-8,8,10,12,16-pentamethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-4-aza-17-oxabicyclo[14.1.0]heptadecane-5,9-dione.

The epothilone derivatives represented by formula I above and processes for their preparation are disclosed in WO 99/02514, WO 99/27890, WO 99/28324. Heretofore, however, there has been no recognition that the subject epothilone derivatives would possess activity in the treatment of tumors resistant to treatment with other known chemotherapeutic agents.

The following are definitions of various terms used to describe the compound represented by formula I above.

The term "alkyl" refers to optionally substituted straight- or branched-chain saturated hydrocarbon groups having from 1 to about 20 carbon atoms, preferably from 1 to about 7 carbon atoms. The expression "lower alkyl" refers to optionally substituted alkyl groups having from 1 to about 4 carbon atoms.

The term "substituted alkyl" refers to an alkyl group substituted by, for example, one to four substituents, such as, halo, trifluoromethyl, trifluoromethoxy, hydroxy, alkoxy, cycloalkyoxy, heterocylooxy, oxo, alkanoyl, aryl, aryloxy, aralkyl, alkanoyloxy, amino, alkylamino, arylamino, aralkylamino, cycloalkylamino, heterocycloamino, disubstituted amino in which the two substituents on the amino group are selected from alkyl, aryl, aralkyl, alkanoylamino, aroylamino, aralkanoylamino, substituted alkanoylamino, substituted arylamino, substituted aralkanoylamino, thiol, alkylthio, arylthio, aralkylthio, cycloalkylthio, heterocyclothio, alkylthiono, arylthiono, aralkylthiono, alkylsulfonyl, arylsulfonyl, aralkylsulfonyl, sulfonamido (e.g., SO_2NH_2), substituted sulfonamido, nitro, cyano, carboxy, carbamyl (e.g., $CONH_2$), substituted carbamyl (e.g., CONH alkyl, CONH aryl, CONH aralkyl or instances where there are two substituents on the nitrogen selected

from alkyl, aryl or aralkyl), alkoxycarbonyl, aryl, substituted aryl, guanidino and heterocyclos, such as, indolyl, imidazolyl, furyl, thienyl, thiazolyl, pyrrolidyl, pyridyl, pyrimidyl and the like. Wherein, as noted above, the substituents themselves are further substituted, such further substituents are selected from the group consisting of halogen, alkyl, alkoxy, aryl and aralkyl. The definitions given herein for alkyl and substituted alkyl apply as well to the alkyl portion of alkoxy groups.

The term "alkenyl" refers to optionally substituted unsaturated aliphatic hydrocarbon groups having from 1 to about 9 carbons and one or more double bonds. Substituents may include one or more substituent groups as described above for substituted alkyl.

The term "halogen" or "halo" refers to fluorine, chlorine, bromine and iodine.

The term "ring system" refers to an optionally substituted ring system containing one to three rings and at least one carbon to carbon double bond in at least one ring. Exemplary ring systems include, but are not limited to, an aryl or a partially or fully unsaturated heterocyclic ring system, which may be optionally substituted.

The term "aryl" refers to monocyclic or bicyclic aromatic hydrocarbon groups having from about 6 to about 12 carbon atoms in the ring portion, for example, phenyl, naphthyl, biphenyl and diphenyl groups, each of which may be substituted.

The term "aralkyl" refers to an aryl group bonded to a larger entity through an alkyl group, such as benzyl.

The term "substituted aryl" refers to an aryl group substituted by, for example, one to four substituents such as alkyl; substituted alkyl, halo, trifluoromethyl, trifluoromethoxy, hydroxy, alkoxy, cycloalkyloxy, heterocyclooxy, alkanoyl, alkanyloxy, amino, alkylamino, dialkylamino, aralkylamino, cycloalkylamino, heterocycloamino, alkanoylamino, thiol, alkylthio, cycloalkylthio, heterocyclothio, ureido, nitro, cyano, carboxy, carboxyalkyl, carbamyl, alkoxycarbonyl, alkylthio, arylthiono, alkylsulfonyl, sulfonamido, aryloxy and the like. The substituent may be further substituted by one or more members selected from the group consisting of halo, hydroxy, alkyl, alkoxy, aryl, substituted alkyl, substituted aryl and aralkyl.

The term "cycloalkyl" refers to optionally substituted saturated cyclic hydrocarbon ring systems, preferably containing 1 to about 3 rings and 3 to about 7 carbon atoms per ring, which may be further fused with an unsaturated C_3–C_7 carbocyclic ring. Exemplary groups include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, cyclodecyl, cyclododecyl, and adamantyl. Exemplary substituents include one or more alkyl groups as described above, or one or more of the groups described above as substituents for alkyl groups.

The terms "heterocycle", "heterocyclic" and "heterocyclo" refer to an optionally substituted, unsaturated, partially saturated, or fully saturated, aromatic or nanoaromatic cyclic group, for example, which is a 4- to 7-membered monocyclic, 7-to 11-membered bicyclic, or 10- to 15-membered tricyclic ring system, which has at least one heteroatom in at least one carbon atom-containing ring. Each ring of the heterocyclic group containing a heteroatom may have 1, 2 or 3 heteroatoms selected from nitrogen atoms, oxygen atoms and sulfur atoms, where the nitrogen and sulfur heteroatoms may also optionally be oxidized and the nitrogen heteroatoms may also optionally be quaternized. The heterocyclic group may be attached at any heteroatom or carbon atom.

Exemplary monocyclic heterocyclic groups include pyrrolidinyl, pyrrolyl, indolyl, pyrazolyl, oxetanyl, pyrazolinyl, imidazolyl, imidazolinyl, imidazolidinyl, oxazolyl, oxazolidinyl, isoxazolinyl, isoxazolyl, thiazolyl, thiadiazolyl, thiazolidinyl, isothiazolyl, isothiazolidinyl, furyl, tetrahydrofuryl, thienyl, oxadiazolyl, piperidinyl, piperizinyl, 2-oxopiperazinyl, 2-oxopiperidinyl, 2-oxopyrrolidinyl, 2-oxazepinyl, azepinyl, 4-piperidonyl, pyridyl, N-oxo-pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, tetrahydropyranyl, tetrahydrothiopyranyl, tetrahydrothiopyranyl sulfone, morpholinyl, thiomorpholinyl, thiomorpholinyl sulfoxide, thiomorpholinyl sulfone, 1,3-dioxolane and tetrahydro-1,1-dioxothienyl, dioxanyl, isothiazolidinyl, thietanyl, thiiranyl, triazinyl, and triazolyl, and the like.

Exemplary bicyclic heterocyclic groups include benzothiazolyl, benzoxazolyl, benzothienyl, quinuclidinyl, quinolinyl, quinolinyl-N-oxide, tetrahydroisoquinolinyl, isoquinolinyl, benzimidazolyl, benzopyranyl, indolizinyl, benzofuryl, chromonyl, coumarinyl, cinnolinyl, quinoxalinyl, indazolyl, pyrrolopyridyl, furopyridinyl (such as furo[2,3-c]pyridinyl, furo[3,1-b]pyridinyl] or furo[2,3-b]pyridinyl), dihydroisoindolyl, dihydroquinazolinyl (such as 3,4-dihydro-4-oxo-quinazolinyl), benzisothiazolyl, benzisoxazolyl, benzodiazinyl, benzofurazanyl, benzothiopyranyl, benzotriazolyl, benzpyrazolyl, dihydrobenzofuryl, dihydrobenzothienyl, dihydrobenzothiopyranyl, dihydrobenzothiopyranyl sulfone, dihydrobenzopyranyl, indolinyl, isochromanyl, isoindolinyl, naphthyridinyl, phthalazinyl, piperonyl, purinyl, pyridopyridyl, quinazolinyl, tetrahydroquinolinyl, thienofuryl, thienopyridyl, thienothienyl, and the like.

Exemplary substituents for the terms "ring system," "heterocycle," "heterocyclic," and "heterocyclo" include one or more substituent groups as described above for substituted alkyl or substituted aryl, and smaller heterocycles, such as, epoxides, aziridines and the like.

The term "alkanoyl" refers to —C(O)-alkyl.

The term "substituted alkanoyl" refers to —C(O)-substituted alkyl.

The term "heteroatoms" shall include oxygen, sulfur and nitrogen.

The compounds represented by formula I form salts with a variety of organic and inorganic acids. Such salts include those formed with hydrogen chloride, hydrogen bromide, methanesulfonic acid, hydroxyethanesulfonic acid, sulfuric acid, acetic acid, trifluoroacetic acid, maleic acid, benzenesulfonic acid, toluenesulfonic acid and various others as are recognized by those of ordinary skill in the art of pharmaceutical compounding. Such salts are formed by reacting a compound represented by formula I in an equivalent amount of the acid in a medium in which the salt precipitates or in an aqueous medium followed by evaporation.

In addition, zwitterions ("inner salts") can be formed and are included within the term "salts" as used herein. Further, solvates and hydrates of the compounds represented by formula I are also included herein.

The compounds represented by formula I above may exist as multiple optical, geometric, and stereoisomers. While the compounds shown herein are depicted for one optical orientation, included within the present invention are all isomers and mixtures thereof.

It is recognized that the compounds represented by formula I above are microtubule-stabilizing agents. Therefore, they are useful in the treatment of a variety of cancers and other proliferative diseases including, but not limited to, the following:

carcinoma, including that of the bladder, breast, colon, kidney, liver, lung, ovary, pancreas, stomach, cervix, thyroid and skin, including aquamous cell carcinoma;

hematopoietic tumors of lymphoid lineage, including leukemia, acute lymphocytic leukemia, acute lymphoblastic leukemia, B-cell lymphoma, T-cell lymphoma, Hodgkins lymphoma, non-Hodgkins lympoma, hairy cell lymphoma and Burketts lymphoma;

hematopoietic tumors of meyloid lineage, including acute and chronic myelogenous leukemias and promyelocytic lumekia;

tumors of mesenchymal origin, including fibrosarcoma and rhabdomyosarcoma;

other tumors, including melanoma, seminoma, teratocarcinoma, neuroblastoma and glioma;

tumors of the central and peripheral nervous system, including astrocytoma, neuroblastoma, glioma, and schwannomas;

tumors of mesenchymal origin, including fibrosarcoma, rhabdomyosarcoma, and osteosarcoma; and

other tumors, including melanoma, xeroderma pigmentosum, keratoacanthoma, seminoma, thyroid follicular cancer and teratocarcincoma.

The foregoing indications are given herein since it cannot be certain which of the named types of tumors, and others as well, may demonstrate resistance to oncology therapy. "Oncology therapy" refers to treatment of cancer or tumors with chemotherapeutic agents that exert a cytotoxic effect in cells. An example of a chemotherapeutic agent is an oncology agent of the taxane family of compounds. It is known, for example, that a considerable number of patients initially responsive to oncology therapy with taxane compounds develop resistance over a course of therapy and that not all cancers respond to treatment with taxane therapy as is the case with virtually all oncology agents. Further, certain diseases, such as cholorectal cancers or melanoma, are known to be innately resistant to taxane therapy.

The subject epothilone compounds are highly potent cytotoxic agents capable of killing cancer cells at low nanometer concentrations and are approximately twice as potent as paclitaxel in inducing tubulin polymerization. More important, the subject compound seem to possess the capacity to retain their antineoplastic activity against human cancers that are naturally insensitive to paclitaxel or have developed resistance to it, both in vitro and in vivo.

Tumors for which the subject epothilone compounds have demonstrated significant antitumor activity include, without intended limitation, the following:

[1] Paclitaxel-resistant—HCT 116VM46 colorectal (multidrug resistant, MDR), Pat-21, breast and Pat-7 ovarian carcinoma (clinical isolates, mechanisms of resistance not fully known), A2780Tax ovarian carcinoma (tubulin mutation);

[2] Paclitaxel-insensitive—Pat-26 human pancreatic carcinoma (clinical isolate) and M5076 murine fibrosarcoma; and

[3] Paclitaxel sensitive—A2780 ovarian, LS 174T and HCT human colon carcinoma.

In addition, the compounds represented by formula I have demonstrated that they are orally efficacious versus preclinical human tumor xenografts grown in immunocompromized mice or rats. Being efficacious upon oral administration is considered a significant advantage of the subject epothilone derivatives.

The present invention thus provides a method of treating a subject, preferably mammals and especially humans, in need of treatment for a tumor that has demonstrated resistance to therapy with the taxane family of oncologic agents, comprising administering to the subject one of the epothilone compounds represented by formula I in an amount effective for such treatment. Other therapeutic agents, such as those described below, may be employed with the subject epothilone compounds in their usual dosages. Such agents may be administered prior to, simultaneously with, or following the subject epothilone compounds.

An effective amount of the epothilone compounds represented by formula I may be determined by one of ordinary skill in the art, and includes exemplary dosage amounts for a human of from about 0.05 to about 200 mg/kg/day. This dosage is typically administered in a single dose, but can be administered in divided doses since the subject compounds are advantageously efficacious via oral administration. The compounds may be administered in a frequent regimen, e.g., every two days for five doses, or intermittently, e.g., every four days for three doses or every eight days for three doses. It will be understood that the specific dose level and frequency of administration for a given subject may be varied and will depend upon a variety of factors, including the subject's age, body weight, general health, sex, diet and the like, the mode of administration if not oral, severity of the condition and the like.

The compounds represented by formula I are administered in pharmaceutical compositions containing an amount thereof effective for cancer therapy, and a pharmaceutically acceptable carrier. Such compositions may contain other therapeutic agents as described below, and may be formulated, for example, by employing conventional solid or liquid vehicles or diluents, as well as pharmaceutical additives of a type appropriate to the mode of desired administration (for example, excipients, binders, preservatives, stabilizers, flavors, etc.) according to techniques such as those well known in the art of pharmaceutical formulation and/or called for accepted pharmaceutical practice.

The compounds represented by formula I may be administered by any suitable means, for example, orally, such as in the form of tablets, capsules, granules or powders; sublingually; bucally; parenterally, such as by subcutaneous, intravenous, intramuscular, or intrasternal injection or infusion techniques (e.g., as sterile injectable aqueous or non-aqueous solutions or suspensions); nasally, such as by inhalation spray; topically, such as in the form of a cream or ointment; or rectally such as in the form of suppositories; in dosage unit formulations containing non-toxic, pharmaceutically acceptable vehicles or diluents. The subject compounds may, for example, be administered in a form suitable for immediate release or extended release. Immediate release or extended release may be achieved by the use of suitable pharmaceutical compositions comprising the present compounds, or, particularly in the case of extended release, by the use of devices such as subcutaneous implants or osmotic pumps. The subject compounds may also be administered liposomally.

Suitable dosage forms for the subject epothilone derivatives include, without intended limitation, a orally effective composition such as a tablet, capsule, solution or suspension containing about 5 to about 500 mg per unit dosage of a compound represented by formula I or a topical form (about 0.01% to about 5% by weight compound represented by formula I, one to five treatments per day). They may be compounded in a conventional manner with a physiologically acceptable vehicle or carrier, excipient, binder, preservative, stabilizer, flavor, etc., or with a topical carrier. The compounds represented by formula I can also be formulated in compositions such as sterile solutions or suspensions for parenteral administration. About 0.1 mg to about 500 mg of a compound represented by formula I may be com-

pounded with a physiologically acceptable vehicle, carrier, excipient, binder preservative, stabilizer, etc., in a unit dosage form as called for by accepted pharmaceutical practice. The amount of active substance in these compositions or preparations is preferably such that a suitable dosage in the range indicated is obtained.

Exemplary compositions for oral administration include suspensions which may contain, for example, microcrystalline cellulose for imparting bulk, alginic acid or sodium alginate as a suspending agent, methylcellulose as a viscosity enhancer, and sweeteners, or flavoring agents such as those known in the art; and immediate release tablets which may contain, for example, microcrystalline cellulose, dicalcium phosphate, starch, magnesium stearate and/or lactose and/or other excipients, binders, extenders, disintegrants, diluents and lubricants such as those known in the art. Molded tablets, compressed tablets or freeze-dried tablets are exemplary forms that may be used. Exemplary compositions include those formulating the present compound(s) with fast dissolving diluents such as mannitol, lactose, sucrose and/or cyclodextrins. Also included in such formulations may be high molecular weight excipients such as celluloses (Avicel) or polyethylene glycols (PEG). Such formulations may also include an excipient to aid mucosal adhesion such as hydroxy propyl cellulose (HPC), hydroxy propyl methyl cellulose (HPMC), sodium carboxy methyl cellulose (SCMC), maleic anhydride copolymer (e.g. Gantrez), and agents to control release such as polyacrylic acid copolymer (e.g. Carbopol 934). Lubricants, glidants, flavors, coloring agents and stabilizers may also be added for ease of fabrication and use.

Exemplary compositions for nasal aerosol or inhalation administration include solutions in saline which may contain, for example, benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, and/or other solubilizing or dispersing agents such as those known in the art.

Exemplary compositions for parenteral administration include injectable solutions or suspensions which may contain, for example, suitable non-toxic, parentally acceptable diluents or solvents, such as Cremophor® (polyoxyethylated caster oil surfactant), mannitol, 1,3-butanediol, water, Ringer's solution, Lactated Ringer's solution, an isotonic sodium chloride solution, or other suitable dispersion or wetting and suspending agents, including synthetic mono- or diglycerides, and fatty acids, including oleic acid. Exemplary compositions for rectal administration include suppositories which may contain, for example, a suitable non-irritating excipient, such as cocoa butter, synthetic glyceride esters or polyethylene glycols, which are solid at ordinary temperature, but liquefy and/or dissolve in the rectal cavity to release the drug.

The compounds of the invention may be administered either alone or in combination with other chemotherapeutic agents or anti-cancer and cytotoxic agents and/or treatments useful in the treatment of cancer or other proliferative diseases. Especially useful are anti-cancer and cytotoxic drug combinations wherein the second drug chosen acts in a different manner or different phase of the cell, e.g., S phase, than the present compounds represented by formula I which exert their effects at the G_2-M phase. Example classes of anti-cancer and cytotoxic agents include, but are not limited to: alkylating agents, such as nitrogen mustards, alkyl sulfonates, nitrosoureas, ethylenimines, and triazenes; antimetabolites, such as folate antagonists, purine analogues, and pyrimidine analogues; antibiotics, such as anthracyclines, bleomycins, mitomycin, dactinomycin, and plicamycin; enzymes, such as L-asparaginase; farnesyl-protein transferase inhibitors; hormonal agents, such as glucocorticoids, estrogens/antiestrogens, androgens/antiandrogens, progestins, and luteinizing hormone-releasing hormone antagonists, octreotide acetate; microtubule-disruptor agents, such as ecteinascidins or their analogs and derivatives; and epothilones A–F or their analogs or derivatives; plant-derived products, such as vinca alkaloids, epipodophyllotoxins, and topoisomerase inhibitors; prenyl-protein transferse inhibitors; and miscellaneous agents such as, hydroxyurea, procarbazine, mitotane, hexamethylmelarnine, platinum coordintination complexes such as cisplatin and carboplatin; and other agents used as anti-cancer and cytotoxic agents such as biological response modifiers, growth factors; immune modulators, and monoclonal antibodies. The subject compounds may also be used in conjunction with radiation therapy.

The compounds represented by formula I may also be formulated or co-administered with other therapeutic agents that are selected for their particular usefulness in administering therapies associated with the aforementioned conditions. For example, the compounds of the invention may be formulated with agents to prevent nausea, hypersensitivity, and gastric irritation, such as antiemetics, and H_1 and H_2 antihistaminics.

The above therapeutic agents, when employed in combination with the compounds of the present invention, may be used in those amounts indicated in the Physicians' Desk Reference (PDR) or as otherwise determined by one of ordinary skill in the art.

The following example is provided, without any intended limitation, to further illustrate the present invention.

EXAMPLE

[1 S-[1R*,3R*(E),7R*,10S*,11R*,12R*,16S*]]-7,11-1-Dihydroxy-8,8,10,12,16-pentamethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-4-aza-17-oxabicyclo[14.1.0] heptadecane-5,9-dione (BMS-247550).

For administration to rodents, the subject compound was administered in either 1:9 ethanol/water, or 1:1:8 Cremphor®/ethanol/water. Final dilution for parenteral administration was made with water one hour before administration. Final dilution for oral administration was made with 0.25 M sodium phosphate buffer. Paclitaxel was dissolved in a 50/50 mixture of ethanol and Cremophor® and maintained at 4° C. Final dilution was made immediately prior to injection to prevent undesirable precipitation. Tumor cell lines HCT 116 human carcinoma and HCT116/V/M46 cells were maintained on McCoy's medium and 10% heat-inactivated fetal bovine serum. A2780 human ovarian carcinoma cells and A2780Tax cells were maintained in IMEM and 10% heat-inactivated fetal bovine serum. All other cell lines were maintained in RPM11640 medium with 10% heat-inactivated fetal bovine serum. Cell lines with acquired resistance will be discussed below.

The in vitro cytotoxicity was assessed in tumor cells by a tetrazolium-based colorimetric assay at 492 mm. The cells were seeded 24 h prior to drug addition. The reagents were added following a 72 h incubation with serially diluted test compound. Measurements were taken after a further three hours incubation. The results are expressed as median cytotoxic concentration (IC_{50} values).

Clonogenic cell colony-formation assay: the potency required for the test compound and paclitaxel to kill clonogenic tumor cells (cells that are able to divide indefinitely to form a colony) in vitro was evaluated by a colony formation assay. The concentration needed to kill 90% of clonogenic cancer cells (IC_{90}) was determined.

Tubulin polymerization assay: the potency required for the test compound and paclitaxel to polymerize tubulin iso-

lated from calf brain was evaluated by published techniques. The effective concentration ($EC_{0.01}$) was defined as the interpolated concentration capable of inducing an initial slope of optical density (OD) of 0.01 OD/minute rate and is calculated using the formula: $EC_{0.01}$=concentration/slope. $EC_{0.01}$ values are expressed as the mean with standard deviation obtained from 3 different concentrations.

In Vivo Antitumor Testing: The following human tumors were used: ovarian carcinoma A2780, A2780Tax and Pat-7 (established from an ovarian tumor biopsy from a patient who had developed resistance to paclitaxel); HCT116, HCT116/VM46 and LS174T colon carcinomas, Pat-21 breast carcinoma, and Pat-26 pancreatic carcinoma (from a liver metastasis biopsy). Pat-7, Pat-21 and Pat-26 xenografts were established initially from primary tumor biopsies directly as xenotransplants grown in whole-body irradiated nude mice without any intervening in vitro cell culturing steps. The innately paclitaxel-insensitive murine fibrosarcoma M5076 was also employed. The human tumor xenografts were maintained in Balb/c nu/nu nude mice. M5076 was maintained in C57BL/6 mice. Tumors were propagated as subcutaneous transplants in the appropriate mouse strain using tumor fragments obtains from donor mice. Tumor passage occupied biweekly for murine tumors and approximately every two to eight weeks for the various human tumor lines. With regard to efficacy testing, M5076 tumors were implanted in (C57B1/6×DBA/2)F1 hybrid mice, and human tumors were implanted in nude mice. All tumor implants for efficacy testing were subcutaneous (sc).

The required number of animals needed to detect a meaningful response (6–8) were pooled at the start of the experiment and each was given a subcutaneous implant of a tumor fragment (~50 mg) with a 13-gauge trocar. For treatment of early-stage tumors, the animals were again pooled before distribution to the various treatment and control groups. For treatment of animals with advanced-stage disease, tumors were allowed to grow to the predetermined size window (tumors outside the range were excluded) and animals were evenly distributed to various treatment and control groups. Treatment of each animal was based on individual body weight. Treated animals were checked daily for treatment related toxicity/mortality. Each group of animals was weighed before the inhalation of treatment (Wt1) and then again following the last treatment dose (Wt2). The difference in body weight (Wt2–Wt1) provided a measure of treatment-related toxicity.

Tumor response was determined by measurement of tumors with a caliper twice a week until the tumors reached a predetermined "target" size of 0.5 or 1.0 g. Tumor weights (mg) were estimated from the formula:

$$\text{Tumor weight}=(\text{length}\times\text{weight})\div 2$$

The maximum tolerated dose (MTD) is defined as the dose level immediately above which excessive toxicity (i.e. more than one death) occurred. The MTD was frequently equivalent to the optimal dose (OD). Activity is described at the OD. Treated mice expiring prior to having their tumors reach target size were considered to have expired from drug toxicity. No control mice expired bearing tumors less than target size. Treatment groups with more than one death caused by drug toxicity were considered to have had excessively toxic treatments and their data were not included in the evaluation of a compound's antitumor efficacy.

Tumor response end-point was expressed in terms of tumor growth delay (T–C value), defined as the difference in time (days) required for the treated tumors (T) to reach a predetermined target size compared to those of the control group (C). A tumor is defined as "cured" when there is no detectable disease at the time of study termination; the interval between study termination and the end of drug treatment always exceeded 10 times the tumor volume doubling time. Group sizes typically consisted of eight mice in all treatment and control groups. Statistical analyses of response data were carried out using the Gehan's generalized Wilcoxon test.

Cytotoxicity Against Cancer Cells in vitro: As shown in FIG. **1**, the results demonstrate the test compound has a broad spectrum of activity against a panel of tumor cell lines in vitro. Of the 21 cells lines tested, the IC_{50} values were in the range of 1.4–34.5 nM. Significantly, the test compound appeared to overcome to a large extent the two main mechanisms of resistance to paclitaxel, viz. MDR resistance due to P-glycoprotein overexpression (exemplified by HCT116/VM46) and β-tubulin mutation (exemplified by A2780Tax). The test compound and paclitaxel were similarly potent in killing clonogenic cells in the two sensitive tumor cell lines (HCT116 and A2780). However, as shown in FIG. **2**, against the three cell lines that had developed resistance to paclitaxel (HCT116/VM46, A2780Tax and Pat-7), the test compound performed far better than paclitaxel, almost completely retaining its cytotoxic potency against these resistant cell lines as compared to the sensitive lines.

Mechanism of Cytotoxicity—Tubulin Polymerization: The cytotoxic activities of the epothilones, like those of the taxanes, have been linked to stabilization of microtubules, which results in mitotic arrest at the G2/M transition. In this regard, the potency of the test compound was about 2.5-fold more potent than paclitaxel.

Mechanism of Cytotoxicity—Effects on Cell Cycle Progression: Similar to paclitaxel, the test compound blocks cells in the mitotic phase of the cell division cycle. Moreover, the concentration of the test compound needed to arrest cells in mitosis, as measured by flow cytometry, corresponds well to the concentration required to kill cells over the same treatment duration. Thus, as shown in FIG. **3**, the test compound at a concentration close to the IC_{90} value (about 7.5 nM, clonogenic cytotoxicity assay) almost completely blocks cells in mitosis as early as 8 hours following the initiation of drug exposure.

Antitumor Activity by Parenteral Administration: The test compound was evaluated in a panel of eight human and murine tumor models, some of which were chosen because of their known, well-characterized resistance to paclitaxel. The tumor model characteristics are shown in Table 1 below. In addition, three paclitaxel-sensitive models were included in order to gain a full assessment of the spectrum of antitumor activity of the test compound.

TABLE 1

Tumor	Histology	Source	Paclitaxel Sensitivity	Resistance Mechanism(s)
Human				
Pat-26	Pancreatic	Biopsy	Insensitive	Unknown
Pat-7	Ovarian	Biopsy	Resistant[1]	MDR[2], MRP[3]
A2780Tax	Ovarian	Cell line	Resistant	Tubulin mutation
HCT116/VM46	Colon	Cell line	Resistant	MDR
Pat-21	Breast	Biopsy	Resistant[1]	Unknown

TABLE 1-continued

Tumor	Histology	Source	Paclitaxel Sensitivity	Resistance Mechanism(s)
A2780	Ovarian	Cell line	Sensitive	NA
HCT116	Colon	Cell line	Sensitive	NA
LS174T	Colon	Cell line	Sensitive	NA
Murine				
M5076	Fibrosarcoma	Cell line	Insensitive	Unknown, Non-MDR

[1]Clinical resistance to Taxol ®
[2]MDR = multidrug resistance due to P-glycoprotein overexpression
[3]MRP = multidrug resistance related protein

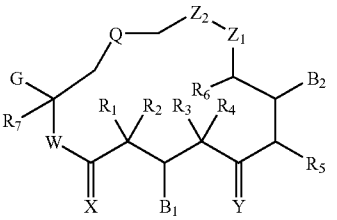
Antitumor Activity by Oral Route of Administration: Since the test compound is more stable at neutral pH than at low pH, the evaluation thereof by oral administration (PO) utilized a pH-buffering vehicle (0.25M potassium phosphate, pH 8.0). Using a every 2 days×5 schedule, the test compound was highly active orally against the Pat-7 human ovarian carcinoma model. In two separate experiments, orally administered test compound yielded 3.1 and 2.5 LCKs at its MTD. In comparison, concomitantly tested IV paclitaxel produced 1.3 and 1.2 LCK, respectively, at its optimal dose and schedule. Paclitaxel is typically inactive when administered by the oral route.

From the foregoing in vitro experimental evidence, it can be seen that the test compound retains its antineoplastic activity in cancer cells that have developed resistance to paclitaxel, whether through overexpression of the MDR P-glycoprotein or tubulin mutation. From the in vivo evidence, the test compound has clearly demonstrated antitumor activity superior to paclitaxel in both paclitaxel-resistant and sensitive tumors, and the murine fibrosarcoma M5076. The test compound was more efficacious than paclitaxel in all five paclitaxel-resistant tumors evaluated in this study (four human and one murine); viz. the clinically-derived paclitaxel resistant Pat-7 ovarian carcinoma; the A2780Tax ovarian carcinoma that is resistant to paclitaxel because of tubulin mutations; the HCT116/VM46 multidrug resistant (MDR) colon carcinoma, the clinically-derived paclitaxel-resistant Pat-21 breast carcinoma; and the murine fibrosarcoma M5076. Against three paclitaxel-sensitive human tumor xenografts, viz. A2780 human ovarians carcinoma; HCT116 and LS 174T human colon carcinoma, the test compound produced antitumor activity equivalent to paclitaxel.

A further advantage of the test compound over the prototypical taxanes is its efficacy by oral administration, producing antitumor activity when given orally that is equivalent to that produced by IV drug administration in two different human tumor xenografts.

What is claimed is:

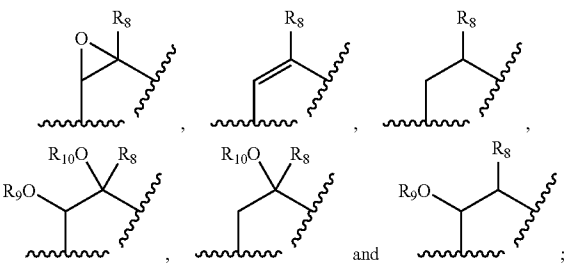
[1. A method for treating a tumor in a mammal, said tumor having demonstrated resistance to oncology therapy, comprising administering to said mammal an effective amount of a composition comprising a pharmaceutically acceptable carrier and an epothilone compound of formula:



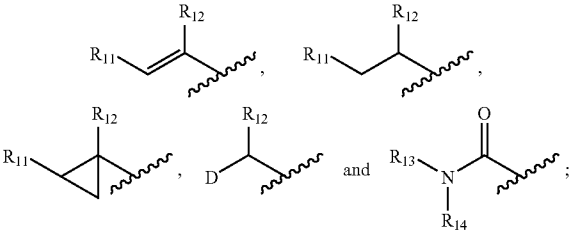
I

wherein:

Q is selected from the group consisting of



G is selected from the group consisting of alkyl, substituted alkyl, aryl, substituted aryl, heterocyclo,



W is O or NR_{15};

X is O or H, H;

Y is selected from the group consisting of O; H, OR_{16}; OR_{17}, OR_{17}; NOR_{18}; H, $NHOR_{19}$; H, $NR_{20}R_{21}$; H, H; and CHR_{22}; wherein OR_{17}, OR_{17} can be a cyclic ketal;

each Z_1 and Z_2 is, independently, selected from the group consisting of CH_2, O, NR_{23}, S, and SO_2, wherein only one of Z_1 and Z_2 can be a heteroatom;

each B_1 and B_2 is, independently, selected from the group consisting of OR_{24}, $OCOR_{25}$, and O—C(═O)—$NR_{26}R_{27}$, and when B_1 is H and Y is OH, H, they can form a six-membered ring ketal or acetal;

D is selected from the group consisting of $NR_{28}R_{29}$, $NR_{30}COR_{31}$ and saturated heterocycle;

each R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_{13}, R_{14}, R_{18}, R_{19}, R_{20}, R_{21}, R_{22}, R_{26} and R_{27} is, independently, selected from the group consisting of H, alkyl, substituted alkyl, and aryl, and when R_1 and R_2 are alkyl can be joined to form a cycloalkyl, and when R_3 and R_4 are alkyl can be joined to form a cycloalkyl;

each R_9, R_{10}, R_{16}, R_{17}, R_{24}, R_{25} and R_{31} is, independently, selected from the group consisting of H, alkyl, and substituted alkyl;

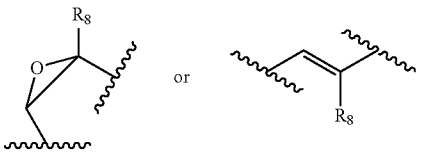
each R_8, R_{11}, R_{12}, R_{28}, R_{30}, R_{32}, and R_{33} is, independently, selected from the group consisting of H, alkyl, substituted alkyl, aryl, substituted aryl, cycloalkyl and heterocyclo; and

each R_{15}, R_{23} and R_{29} is, independently, selected from the group consisting of H, alkyl, substituted alkyl, aryl, substituted aryl, cycloalkyl, heterocyclo, $R_{32}C{=}O$, $R_{33}SO_2$, hydroxy, O-alkyl or O-substituted alkyl;

and pharmaceutically acceptable salts thereof and any hydrates, solvates or geometric, optical and stereoisomers thereof,

with the proviso that compounds wherein W and X are both O; R_1, R_2 and R_7 are H; R_3 R_4 and R_6 are methyl; R_8 is H or methyl; Z_1 and Z_2 are CH_2; G is 1-methyl-2-(substituted-4-thiazolyl)ethenyl; and Q is as defined above, are excluded.]

[2. The method of claim 1 wherein Q is



X is O; Y is O; each Z_1 and Z_2 is, independently, CH_2; and W is NR_{15}.]

[3. The method of claim 1 wherein said epothilone compound is selected from the group consisting of

[1S-[1R*,3R*(E), 7R*,10S*,11R*,12R*,16S*]]-7,11-dihydroxy-8,8,10,12,16-pentamethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-4,13,17-trioxabicyclo[14.1.0]heptadecane-5,9-dione;

[1S-[1R*,3R*(E),7R*,10S*,11R*,12R*,16S*]]-7,11-dihydroxy-8,8,10,12-tetrametlnrl-3-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-4,13,17-trioxabicyclo[14.1.0]heptadecane-5,9-dione;

[4S-[4R*,7S*,8R*,9R*,15R*(E)]]-4,8-dihydroxy-5,5,7,9,13-pentamethyl-16-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-1,10-dioxa-13-cyclohexadecene-2,6-dione;

[4S-[4R*,7S*,8R*,9R*,15R*(E)]]-4,8-dihydroxy-5,5,7,9-tetramethyl-16-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-1,10-dioxa-13-cyclohexadecene-2,6-dione;

[1S-[1R*,3R*(E),7R*,10S*,11R*,12R*,16S*]]-7,11-dihydroxy-8,8,10,12,16-pentamethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-4,14,17-trioxabicyclo[14.1.0]heptadecane-5,9-dione;

[1S-[1R*,3R*(E),7R*,10S*,11R*,12R*,16S*]]-7,11-dihydroxy-8,8,10,12-tetramethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-4,14,17-trioxabicyclo[14.1.0]heptadecane-5,9-dione;

[4S-[4R*,7S*,8R*,9R*,15R*(E)]]-4,8-dihydroxy-5,5,7,9,13-pentamethyl-16-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-1,11-dioxa-13-cyclohexadecene-2,6-dione;

[4S-[4R*,7S*,8R*,9R*,15R*(E)]]-4,8-dihydroxy-5,5,7,9-tetramethyl-16-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-1,11-dioxa-13-cyclohexadecene-2,6-dione;

[1S-[1R*,3R*(E),7R*,10S*,11R*,12R*,16S*]]-7,11-dihydroxy-8,8,10,12,16-pentamethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-4,17-dioxabicyclo[14.1.0]heptadecane-9-one;

[1S-[1R*,3R*(E),7R*,10S*,11R*,12R*,16S*]]-7,11-dihydroxy-8,8,10,12-tetramethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-4,17-dioxabicyclo[14.1.0]heptadecane-9-one;

[1S-[1R*,3R*(E),7R*,10S*,11R*,12R*,16S*]]-7,11-dihydroxy-3,8,8,10,12,16-hexamethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-4,17-dioxabicyclo[14.1.0]heptadecane-5,9-dione;

[1S-[1R*,3R*(E),7R*,10S*,11R*,12R*,16S*]]-7,11-dihydroxy-3,8,8,10,12-pentamethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-4,17-dioxabicyclo[14.1.0]heptadecane-5,9-dione;

[4S-[4R*,7S*,8R*,9R*,15R*(E)]]-4,8-dihydroxy-5,5,7,9,13,16-hexamethyl-16-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-1-oxa-13-cyclohexadecene-2,6-dione;

[4S-[4R*,7S*,8R*,9R*,15R*(E)]]-4,8-dihydroxy-5,5,7,9,16-pentamethyl-16-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-1-oxa-13-cyclohexadecene-2,6-dione;

[1S-[1R*,3R*(E),7R*,10S*,11R*,12R*,16S*]]-7,11-dihydroxy-8,8,10,12,16-pentamethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-4,17-dioxabicyclo[14.1.0]heptadecane-5,9-dione;

[1S-[1R*,3R*(E),7R*,10S*,11R*,12R*,16S*]]-7,11-dihydroxy-6,8,8,10,12-pentamethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-4,17-dioxabicyclo[14.1.0]heptadecane-5,9-dione;

[1S-[1R*,3R*(E),7R*,10S*,11R*,12R*,16S*]]-7,11-dihydroxy-8,8,10,12,16-pentamethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-4-aza-17-oxabicycllo[14.1.0]heptadecane-5,9-dione;

[1S-[1R*,3R*(E),7R*,10S*,11R*,12R*,16S*]]-7,11-dihydroxy-8,8,10,12-tetramethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-4-aza-17oxabicyclo[14.1.0]heptadecane-5,9-dione;

[4S-[4R*,7S*,8R*,9R*,15R*(E)]]-4,8-dihydroxy-5,5,7,9,13-pentamethyl-16-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-1-aza-13-cyclohexadecene-2,6-dione;

[4S-[4R*,7S*,8R*,9R*,15R*(E)]]-4,8-dihydroxy-5,5,7,9-tetramethyl-16-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-1-aza-13-cyclohexadecene-2,6-dione;

[1S-1R*,3R*(E),7R*,10S*,11R*,12R*,16S*]]-7,11-dihydroxy-4,8,8,10,12,16-hexamethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-4-aza-17-oxabicyclo[14.1.0]heptadecane-5,9-dione;

[1S-[1R*,3R*(E),7R*,10S*,11R*,12R*,16S*]]-7,11-dihydroxy-4,8,8,10,12-pentamethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-4-aza-17-oxabicyclo[14.1.0]heptadecane-5,9-dione;

[4S-[4R*,7S*,8R*,9R*,15R*(E)]]-4,8-dihydroxy-1,5,5,7,9,13-hexamethyl-16-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-1-aza-13-cyclohexadecene-2,6-dione;

[4S-[4R*,7S*,8R*,9R*,15R*(E)]]-4,8-dihydroxy-1,5,5,7,9-pentamethyl-16-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-1-aza-13-cyclohexadecene-2,6-dione;

[1S-[1R*,3R*(E),7R*,10S*,11R*,12R*,16S*]]-7,11-dihdyroxy-8,8,10,12,16-pentamethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-13-aza-4,17dioxabicyclo[14.1.0]heptadecane-5,9-dione;

[1S-[1R*,3R*(E),7R*,10S*,11R*,12R*,16S*]]-7,11-dihydroxy-8,8,10,12-tetramethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-13-aza-4,17dioxabicyclo[14.1.0]heptadecane-5,9-dione;

[4S-[4R*,7S*,8R*,9R*,15R*(E)]]-4,8-dihydroxy-5,5,7,9,13-pentamethyl-16-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-10-aza-1-oxa-13-cyclohexadecene-2,6-dione;

[4S-[4R*,7S*,8R*,9R*,15R*(E)]]-4,8-dihydroxy-5,5,7,9-tetramethyl-16-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-10-aza-1-oxa-13-cyclohexadecene-2,6-dione;

[1S-[1R*,3R*(E),7R*,10S*,11R*,12R*,16S*]]-7,11-dihydroxy-8,8,10,12,16-pentamethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-14-aza-4,17-dioxabicyclo[14.1.0]heptadecane-5,9-dione;

[1S-[1R*,3R*(E),7R*,10S*,11R*,12R*,16S*]]-7,11-dihydroxy-8,8,10,12-tetramethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-14-aza-4,17-dioxabicyclo[14.1.0]heptanedecane-5,9-dione;

[4S-[4R*,7S*,8R*,9R*,15R*(E)]]-4,8-dihydroxy-5,5,7,9,13-pentamethyl-16-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-11-aza-1-oxa-13-cyclohexadecene-2,6dione;

[4S-[4R*,7S*,8R*,9R*,15R*(E)]]-4,8-dihydroxy-5,5,7,9-tetramethyl-16-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-11-aza-1-oxa-13-cyclohexadecene-2,6-dione;

[1S-[1R*,3R*,7R*,10S*,11R*,12R*,16S*]]-N-phenyl-7,11-dihydroxy-8,8,10,12,16-pentamethyl-5,9-dioxo-4,17-dioxabicyclo[14.1.0]heptadecane-3-carboxamide;

[1S-[1R*,3R*,7R*,10S*,11R*,12R*,16S*]]-N-phenyl-7,11-dihydroxy-8,8,10,12-tetramethyl-5,9-dioxo-4,17-dioxabicyclo[14.1.0]heptadecane-3-carboxamide;

[4S-[4R*,7S*,8R*,9R*,15R*]]-N-phenyl-4,8-dihydroxy-5,5,7,9,13-pentamethyl-2,6-dioxo-1-oxa-13-cyclohexadecene-16-carboxamide;

[4S-[4R*,7S*,8R*,9R*,15R*]]-N-phenyl-4,8-dihydroxy-5,5,7,9-tetramethyl-2,6-dioxo-1-oxa-13-cyclohexadecene-16-carboxamide;

[1S-[1R*,3R*(E),7R*,10S*,11R*,12R*,16S*]]-7,11-dihydroxy-8,8,10,12,16-pentamethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)cyclopropyl]-4,17-dioxabicyclo[14.1.0]heptadecane-5,9-dione;

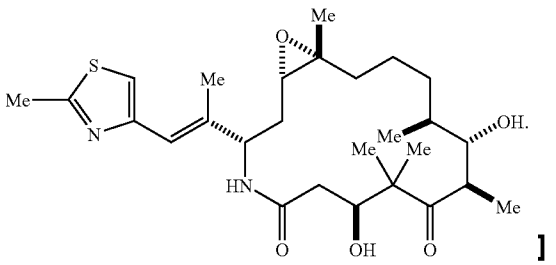
[1S-[1R*,3R*(E),7R*,10S*,11R*,12R*,16S*]]-7,11-dihydroxy-8,8,10,12-tetramethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)cyclopropyl]-4,17-dioxabicyclo[14.1.0]heptadecane-5,9-dione; and

[4S-[4R*,7S*,8R*,9R*,15R*(E)]]-4,8-dihydroxy-5,5,7,9,13-pentamethyl-16-[1-methyl-2-(2-hydroxymethyl-4-thiazolyl)ethenyl]-1-aza-13(Z)-cyclohexadecene-2,6-dione;

and pharmaceutically acceptable salts, solvates and hydrates thereof.]

[4. The method of claim **1** wherein said epothilone compound is of formula:

[



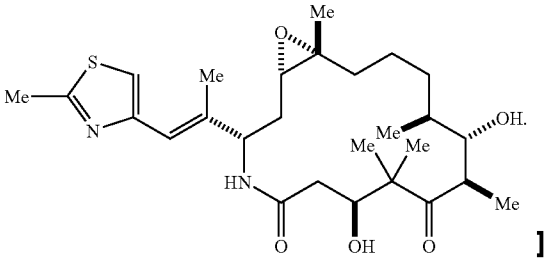
]

[5. The method of claim **1** wherein said mammal is a human.]

[6. The method of claim **1** wherein the composition containing said epothilone compound is administered parenterally.]

[7. The method of claim **6** wherein said epothilone compound is of formula:

[



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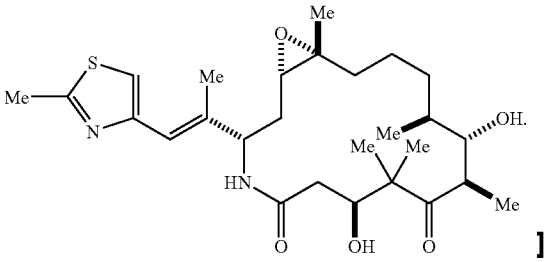
[8. The method of claim **1** wherein the composition containing said epothilone compound is administered orally.]

[9. The method of claim **8** wherein said epothilone compound is of formula:

[



]

[10. The method of claim **1** wherein said tumor was initially not responsive to oncology therapy.]

[11. The method of claim **1** wherein said tumor was initially responsive to oncology therapy, but developed resistance thereto during the course of treatment.]

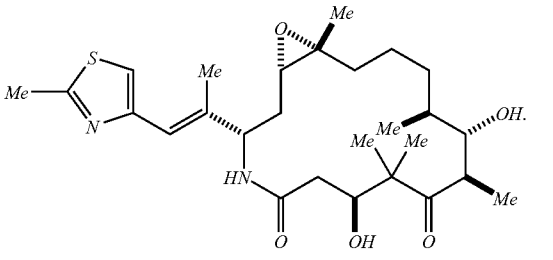
[12. The method of claim **1** wherein said compound is administered simultaneously or sequentially with a chemotherapeutic agent useful in the treatment of cancer or other proliferative diseases.]

[13. The method of claim **1** wherein the oncology therapy is a taxane.]

[14. The method of claim **1** wherein the oncology therapy is paclitaxel.]

[15. The method of claim **1** wherein the tumor is of the bladder, breast, colon, kidney, liver, lung, ovary, pancreas, stomach, cervix, thyroid or skin.]

16. A method for treating a tumor in a mammal, said tumor being resistant to oncology therapy with a taxane, comprising administering to said mammal an effective amount of a composition comprising a pharmaceutically acceptable carrier and a compound having the formula,



17. The method of claim 16 wherein said mammal is a human.

18. The method of claim 17 wherein said tumor was initially not responsive to taxane therapy.

19. The method of claim 17, wherein said tumor was initially responsive to taxane therapy, but developed resistance thereto during the course of treatment.

20. The method of claim 17, wherein said tumor is innately resistant to taxane therapy.

21. The method of claim 17, wherein the taxane is paclitaxel.

22. The method of claim 17 wherein the tumor is of the bladder, breast, colon, kidney, liver, lung, ovary, pancreas, stomach, cervix, thyroid or skin.

23. The method of claim 22, wherein the tumor is of the breast.

24. The method of claim 22, wherein the tumor is of the pancreas.

25. The method of claim 23, wherein the oncology therapy is paclitaxel.

26. The method of claim 23 wherein said tumor was initially not responsive to taxane therapy.

27. The method of claim 23 wherein said tumor was initially responsive to taxane therapy, but developed resistance thereto during the course of treatment.

28. The method of claim 23, wherein said tumor is innately resistant to taxane therapy.

* * * * *